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# cancer treatment reports

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*The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.*

# Effects of High-Dose Methotrexate and Vincristine on Ovarian and Testicular Functions in Patients Undergoing Postoperative Adjuvant Treatment of Osteosarcoma [1]

Robert C. Shamberger, Steven A. Rosenberg, Claudia A. Seipp, and Richard J. Sherins [2,3,*]

## SUMMARY

**We have studied gonadal function in five patients (three men and two women) who received high-dose methotrexate (HD-MTX) alone and in 12 patients (seven men and five women) who received HD-MTX and vincristine (VCR) as postoperative adjuvant therapy for osteosarcoma. Testicular function was assessed by determination of testicular size, sperm concentration, and serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels while evaluation of ovarian function was based on menstrual history and serum FSH, LH, estradiol, and progesterone levels. The two women who received HD-MTX and all five women who received HD-MTX and VCR had regular cyclic menses not only after therapy but also during treatment. Serum FSH, LH, estradiol, and progesterone levels were normal in all women studied. Among the ten men studied, testicular function was normal in the six patients evaluated initially several months after the completion of therapy. In contrast, severe oligospermia was noted among four men evaluated during and immediately after treatment. From serum samples obtained before, during, and after treatment we have determined that approximately one half of the men who received HD-MTX alone or in combination with VCR develop transient testicular failure associated with a significant increase in serum FSH but not LH levels. Sperm concentration and serum FSH levels then return to normal after completion of chemotherapy.**

[Cancer Treat Rep 65:739–746, 1981]

Osteosarcoma is a disease of the young, with 40%–50% of patients < 20 years of age (1,2). Current therapy of these patients involves amputation of the extremity often followed by adjuvant chemotherapy with high-dose methotrexate (HD-MTX) and vincristine (VCR) (3–6). Because of the young age of these patients and their expected longevity if cure is achieved, attention should be given to the long-term complications of treatment.

The risk of sterility due to chemotherapy is an area of concern to many patients and their families. Gonadal injury in man is drug- and dose-dependent and has varied markedly among treatment regimens. Alkylating agents (7–27)[4] and methylhydrazines (28–33) are known to cause gonadal damage. However, no previous studies have investigated the potential for gonadal injury in patients receiving

[1]Received Aug 7, 1980; revised Feb 17, 1981; accepted Mar 4, 1981.

[2]Surgery Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute (*R. C. Shamberger, S. A. Rosenberg,* and *C. A. Seipp*) and Developmental Endocrinology Branch, Intramural Research Program, National Institute of Child Health and Human Development (*R. J. Sherins*), Bethesda, MD.

[3]We are grateful to Dr. John L. Ziegler for his encouragement in this study. We also thank Mr. David R. Brightwell and Ms. Natasha Norman for their technical assistance.

**Reprint requests to*: Richard J. Sherins, MD, Developmental Endocrinology Branch, National Institute of Child Health and Human Development, Bldg 10, Rm B09, National Institutes of Health, Bethesda, MD 20205.

[4]Shamberger RC, Sherins RJ, Ziegler JL, et al. The effects of postoperative adjuvant chemotherapy and radiotherapy on ovarian function in women undergoing treatment for soft tissue sarcoma. JNCI. In press.

TABLE 1.—Women treated with adjuvant chemotherapy for osteosarcoma

| Patient No. | Age at therapy (yrs) | No. of pregnancies: Before therapy | No. of pregnancies: After therapy | Interval after therapy (mos) | FSH* (mIU/ml) | LH* (mIU/ml) | Cycles of therapy | MTX (g) | VCR (mg) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Women after HD-MTX* | | | | | |
| 1 | 15 | 0 | 0 | 30 | 11.1 | 17.5 | 26 | 412 | 0 |
| 2 | 31 | 0 | 0 | 35 | 8.2 | 4.2 | 26 | 250 | 0 |
| | | | | *Women after HD-MTX and VCR* | | | | | |
| 3 | 20 | 0 | 0 | 11 | 13.6 | 13.8 | 21† | 291 | 42 |
| 4 | 29 | 2 | 0 | 14 | 11.7 | 11.0 | 9† | 97 | 18 |
| | | | | *Women receiving HD-MTX and VCR* | | | | | |
| 5 | 13 | 0 | 0 | — | 15.2 | 21.8 | 11–14‡ | 145 | 28 |
| 6 | 17 | 0 | 1§ | — | 19.2 | 12.3 | 14–26‡ | 354 | 52 |
| 7 | 28 | 1 | 0 | — | 17.1 | 12.7 | 19–26‡ | 310 | 52 |

*Mean value of samples drawn during evaluation either during or after therapy.
†Patients stopped treatment before completion of entire protocol.
‡Cycles of therapy received during evaluation.
§Patient became pregnant within 4 mos of completion of therapy and pregnancy eventuated in the birth of a normal child.

HD-MTX. Accordingly, we have evaluated gonadal function among patients undergoing adjuvant treatment of osteosarcoma to assess the toxicity of this agent on human gonadal tissue.

## PATIENTS

All patients in this study were receiving therapy for osteosarcoma of an extremity using protocols established in 1975 by the Surgery Branch of the National Cancer Institute (NCI), Bethesda, MD (6). Patients treated initially in the NCI study had received only HD-MTX but patients who entered the treatment study at a later phase received VCR in addition to HD-MTX.

At the time the study was begun, 11 patients had completed chemotherapy: 12–36 months previously (seven men) or 11–35 months previously (four premenopausal women). Six additional patients (three men and three women) were studied while receiving adjuvant chemotherapy. A summary of patient information is given in tables 1 and 2. No patient had a history of infertility or abnormal pubertal development. All patients agreed to participate in this study designed to test the functional integrity of the ovary or testis.

All patients underwent amputation of the involved extremity. Five patients (three men and two women) received HD-MTX with leucovorin rescue, while 12 patients (seven men and five women) re-

TABLE 2.—Men treated with adjuvant chemotherapy for osteosarcoma

| Patient No. | Age at therapy (yrs) | No. of children: Before therapy | No. of children: After therapy | Interval after therapy (mos) | FSH* (mIU/ml) | LH* (mIU/ml) | Testosterone* (ng/dl) | Sperm concentration (cells × $10^6$/ml) | Cycles of therapy | MTX (g) | VCR (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Men after HD-MTX* | | | | | | |
| 1 | 13 | 0 | 0 | 36 | 16.4 | 4.5 | 300 | — | 26 | 132 | 0 |
| 2 | 16 | 0 | 0 | 34 | 11.0 | 12.8 | 382 | 21.0 | 26 | 389 | 0 |
| 3 | 40 | 2 | 0 | 32 | 10.3 | 6.7 | 399 | 60.0 | 23 | 373 | 0 |
| | | | | | *Men after HD-MTX and VCR* | | | | | | |
| 4 | 14 | 0 | 0 | 14 | 6.8 | 6.5 | 627 | 45.0 | 26 | 295 | 52 |
| 5 | 22 | 0 | 0 | 28 | 11.7 | 12.7 | 745 | 85.0 | 26 | 357 | 52 |
| 6 | 24 | 0 | 0 | 19 | 17.4 | 8.4 | 470 | 39.9 | 26 | 407 | 52 |
| 7 | 27 | 2 | 0 | 12 | 30.5 | 14.2 | 453 | 11.3 | 26 | 413 | 52 |
| | | | | | *Men receiving HD-MTX and VCR* | | | | | | |
| 8 | 20 | 0 | — | — | 5.5 | 12.6 | 639 | 0.5 | 13–26† | 458 | 52 |
| 9 | 24 | 0 | — | — | 23.4 | 10.5 | 768 | 0.6 | 17–26† | 367 | 52 |
| 10 | 44 | 1 | — | — | 16.2 | 11.2 | 389 | 0.8 | 13–18† | 307 | 34 |

*Mean values of samples obtained during evaluation either during or after therapy.
†Cycles of therapy received during evaluation.

ceived VCR in addition to HD-MTX. The initial MTX dose was 50 or 100 mg/kg and this was increased to a maximum of 250 mg/kg over two to five courses and was maintained at this level. MTX was given as a 6-hour infusion followed in 2 hours by leucovorin (15 mg/m²) that was administered iv every 6 hours, for a total of eight doses. All patients were vigorously hydrated with 3000 ml/m²/day containing 300 mEq of sodium bicarbonate/m²/day beginning 12 hours prior to the MTX infusion and continuing for 24 hours from the start of the infusion. MTX was readministered every 3 weeks as described above. The total course of treatment included 26 cycles lasting 36 months. Two milligrams of VCR was given iv ½ hour prior to the MTX infusion in those patients who received both agents.

## STUDY DESIGN

Patients participating in the study who had completed therapy were initially evaluated as outpatients and were followed at 2- or 3-month intervals at the time of their routine clinic visits. Patients who were in the process of receiving adjuvant therapy were followed at 3-week intervals coincident with their hospitalizations.

At the initial evaluation, a detailed history was obtained to document whether any sexual dysfunction or history of infertility was present before or after treatment. Physical examination of the men included evaluation of testicular size and an assessment of the genitalia and breasts for abnormalities. Testicular length was measured in both the longitudinal and transverse axes and the volume was estimated by comparing size with a series of ovoids of calibrated volume. In women a pelvic examination was included.

Serum gonadotropin and sex steroid levels were studied to further assess gonadal function. Two blood specimens were drawn at 30-minute intervals at each clinic visit or hospital admission and the serum was rapidly separated and frozen for subsequent assay of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (men) or estradiol and progesterone (women) by specific radioimmunoassay methods (34–38). For comparison, FSH and LH values were also obtained from a group of 41 normal men (18–48 years old) of proven fertility who had ejaculates with > 20 million sperm/ml and > 60% oval forms. Testosterone levels were obtained in 26 of these men. From among the normal volunteers the mean concentrations ± SD were: FSH, 11.8 ± 5.5 mIU/ml; LH, 8.7 ± 2.7 mIU/ml; and testosterone, 523 ± 171 ng/dl. Fifteen women with normal cyclic menses had blood specimens drawn daily throughout their menstrual cycles to provide the normal levels by menstrual phase as shown in table 3, and 100 postmenopausal women provided blood samples to serve as normative data for women who had spontaneously ceased menstruating.

Among the male subjects sperm output was assessed from ejaculates collected monthly by masturbation or condom technique after a 24-hr period of abstinence. Specimens were frozen until the next clinic visit or admission. Sperm count was determined using a hemocytometer, and cellular morphology was examined from smears of the seminal fluid (39).

Detailed records of menstrual cycles were maintained by the women to document cyclic menses and also to correlate hormone levels with the phase of menstrual cycles in which they were obtained.

The time course of gonadal injury in patients could also be evaluated retrospectively because numerous frozen serum samples were available from blood drawn before, during, and after treatment and stored at –180°C in the serum bank of the Surgery Branch, NCI.

Statistical analysis of the data was performed using Student's unpaired t test and values are expressed in the text as mean ± SD.

## RESULTS

### Ovarian Function

Four women (patients 1–4) were studied after completion of therapy; all reported regular cyclic menses at the time of their first evaluation. None of the four women had developed amenorrhea or menstrual irregularities even while receiving treatment. Two of the four women (patients 1 and 2) received HD-MTX alone and two (patients 3 and 4) received HD-MTX and VCR. The continuation of cyclic menses during treatment suggested little or no ovarian toxicity from these agents. Three additional women (patients 5–7) were studied while receiving chemotherapy and each reported regular cyclic menses during their treatment. Serum FSH and LH levels obtained either in the follicular or luteal phase of the menstrual cycle were normal in all seven women studied (fig 1). Serum estradiol and progesterone levels were similarly appropriate for the phase of the cycle in which they were obtained (data not shown).

### Testicular Function Evaluated After Completion of Therapy

Three men (patients 1–3) were studied only after having completed chemotherapy with HD-MTX.

TABLE 3.—Range of serum FSH, LH, and estradiol levels among 15 normal premenopausal and 100 normal postmenopausal women

| Menstrual phase | FSH (mIU/ml) | LH (mIU/ml) | Estradiol (pg/ml) |
|---|---|---|---|
| Follicular | 5–15 | 6–27 | 40 |
| Midcycle | 7–14 | 45–154 | 170–770 |
| Luteal | 9–27 | 5–17 | 190–340 |
| Postmenopausal | 35–217 | 35–96 | < 40 |

Their testicular volumes (20, 20, and 20 ml) were within the normal range (16–32 ml) (41), and sperm concentration was normal (> 20 million/ml) in the two men (patients 2 and 3) who provided ejaculates (fig 2). The mean serum FSH, LH, and testosterone levels were within the normal range (figs 3–5).

Four additional men (patients 4–7) who had completed chemotherapy at the time of initial evaluation had received HD-MTX and VCR. Their testicular volumes (17.5, 20, 25, and 25 ml) were within the normal range, and sperm concentration was > 20 million/ml in three of the men. In the fourth man (patient 7), however, the initial sperm concentration was only 2.6 million/ml when first evaluated 1 month after completion of therapy. The concentration then progressively increased during the study period to a level of 21 million/ml 15 months after treatment. Simultaneously, his FSH concentration (40 mIU/ml), which was increased above the normal range (4–25 mIU/ml), decreased to 22 mIU/ml, a value within the normal range. The other men had normal serum FSH values. All four men had normal serum LH and testosterone concentrations.

## Testicular Function Evaluated During Therapy

Three men were initially evaluated late in the course of chemotherapy. All three patients received HD-MTX and VCR. Their testicular volumes (25, 27.5, and 27.5 ml) were normal but mean sperm concentration was only 0.5–0.8 million/ml. Nevertheless, the mean serum FSH, LH, and testosterone levels (fig 2) were normal at that time.

## Longitudinal Studies

Because of the reduced sperm output in the three men studied during the administration of MTX and VCR, serum FSH and LH concentrations were measured from numerous frozen serum samples obtained prior to, during, and after treatment in all seven men who had completed therapy to evaluate the possibility of earlier transient gonadal injury. All samples were measured in a single assay to avoid interassay variation. In two of the three men who received HD-MTX alone, serum FSH and LH levels remained within the normal range throughout therapy as illustrated in figure 6A. In the third patient, however, serum FSH levels increased significantly ($P < 0.01$) early in the course of therapy but returned to normal after completion of treatment (fig 6B).

Of the four men who had completed HD-MTX and VCR therapy, gonadotropin levels remained normal in two patients (fig 6C), but FSH concentration increased within the normal range in a third man as

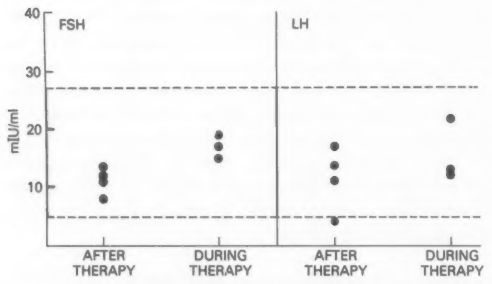


FIGURE 1.—Mean serum FSH and LH values obtained from women either while receiving chemotherapy or after completion of treatment. Shaded area = range of normal values of samples drawn during the follicular and luteal phases.

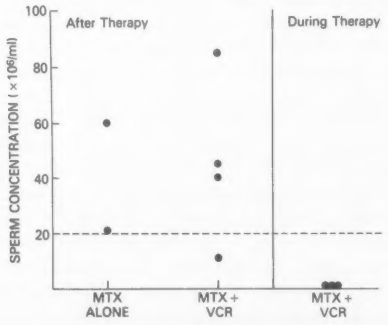


FIGURE 2.—Mean sperm concentration determined for each patient among the various treatment groups. Dashed line = lower limit of normal for our lab (20 million/ml).

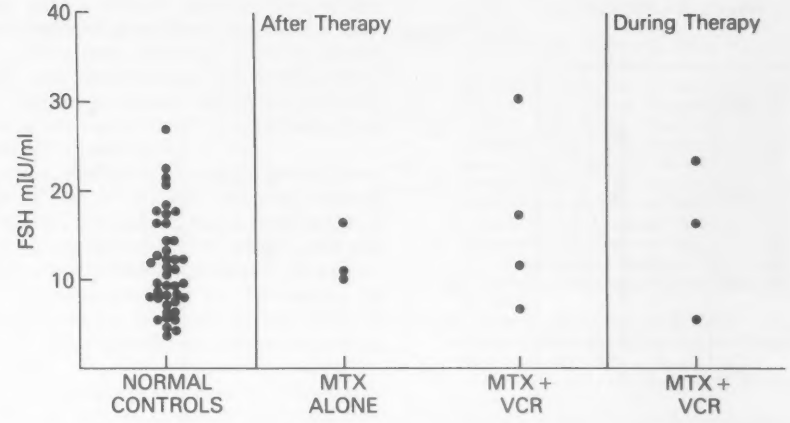


FIGURE 3.—Mean serum FSH levels determined for each patient among the various treatment groups. Data of normal age-matched controls are shown on the left.

therapy progressed and his FSH levels remained elevated above baseline 19 months after therapy (data not shown). The fourth man previously discussed showed a marked elevation in FSH levels during therapy ($P < 0.001$), which returned within the normal range 15 months after treatment, although the concentration remained above his baseline value (fig 6D). Serum LH concentrations in all seven men were unchanged throughout therapy.

## DISCUSSION

In assessing potential adverse effects of chemotherapeutic agents on gonadal function, attention must be given to which agent is used and the dose that is administered. There is no information regarding the effect of high doses of MTX on human gonadal function. Data available from treatment of women with choriocarcinoma demonstrate that there are no adverse effects on the ovary at much lower doses of MTX. The availability of a group of young patients who received HD-MTX for osteosarcoma provided the opportunity to assess the effect of higher doses of this drug on gonadal function.

We have found no evidence of ovarian dysfunction when MTX is administered alone, even at doses of 250 mg/kg given every 3 weeks for 36 months. In contrast, some men develop testicular dysfunction

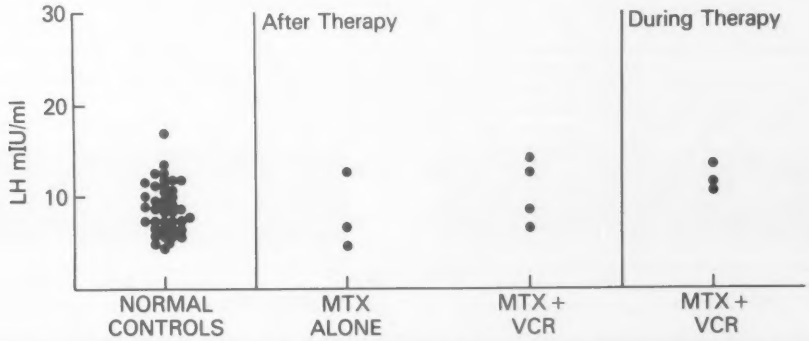


FIGURE 4.—Mean serum LH levels determined for each patient among the various treatment groups. Data of normal age-matched controls are shown on the left.

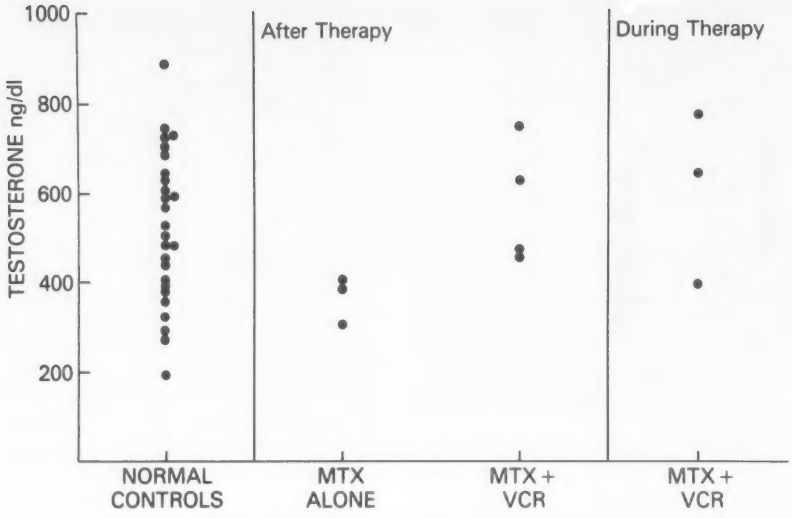


FIGURE 5.—Mean serum testosterone levels determined for each patient among the various treatment groups. Data of normal age-matched controls are shown on the left.

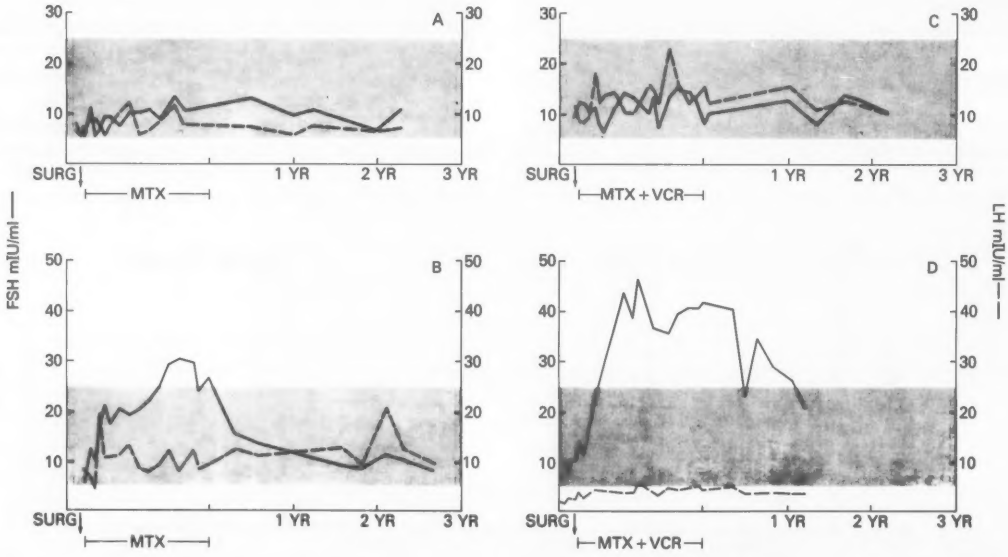


FIGURE 6.—FSH and LH levels measured in serial blood samples drawn before, during, and after treatment; representative profiles are shown. Patients A and B received HD-MTX alone while patients C and D received HD-MTX and VCR. Intervals of treatment are shown by horizontal bars. Time following completion of chemotherapy is also indicated.

when treated with a similar regimen. One of the three men showed transient elevation in FSH during therapy, with return to normal levels promptly after therapy was discontinued; 34 months after completion of treatment sperm output was normal. Similar findings are noted among the patients who received both MTX and VCR.

In the four men studied after completion of treatment with MTX and VCR, only one man showed an increase in FSH level above the normal range in association with decreased sperm output, and his testicular dysfunction returned to normal during our observation. Oligospermia was evident among all three men studied during drug therapy and this was also associated with a significant increase in serum FSH in one man.

Our data are in keeping with results from experimental studies in rodents where MTX or aminopterin (both folic acid antagonists) produced inhibition of spermatogenesis (41,42). Although the animals were infertile during therapy, most recovered after cessation of treatment. MTX is an antimetabolite which is cell cycle-specific for S phase. It competitively binds to dihydrofolate reductase interfering with folic acid reduction to tetrahydrofolic acid which is essential for biosynthesis of thymidylic acid. Since it is active only against cells in the S phase synthesizing DNA, it would not be expected to destroy the slowly dividing spermatogonial stem cell population but rather produce a suppression of that population and of spermatogenesis as was found to occur. In studies of VCR a transient dose-dependent decrease in fertility rate has also been demonstrated (43,44). Histologic evaluation of the testes showed loss of pachytene spermatocytes but no spermatogonial stem cell-killing (45,46). VCR impairs thymidine incorporation into primary spermatogonia (44). It also produces metaphase arrest by crystallization of microtubular and spindle proteins and is unlikely to produce spermatogonial stem cell-killing. This was confirmed in both the experimental and our human studies.

On an empiric basis neither agent would be expected to produce injury in the nondividing ovum and although no experimental studies on the effect of MTX or VCR on ovarian function have been reported, we demonstrated no effect in our human population.

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# Chemotherapy for Colorectal Cancer With a Combination of PALA and 5-FU[1,2]

**Agop Y. Bedikian,* John R. Stroehlein, David A. Karlin, Rowland W. Bennetts, Gerald P. Bodey, and Manuel Valdivieso[3]**

## SUMMARY

**Fifty-one previously untreated patients with advanced, measurable colorectal cancer were randomized to receive single doses of PALA and 5-FU either weekly (24 patients) or daily for 5 days every 4 weeks (27 patients). In both schedules the daily dose of PALA was administered iv over 1 hour, while the dose of 5-FU was administered iv over 30 minutes, starting 3 hours after completion of the PALA dose. Doses of both drugs were changed simultaneously, based on toxic effects. Partial responses were seen in four of 24 evaluable patients receiving the weekly regimen and in three of 26 patients receiving the 5-day regimen. Skin rash, mucositis, and diarrhea were the dose-limiting toxic effects in both treatment regimens. These toxic effects were more common and severe at higher doses and on the weekly schedule. Myelosuppression was mild to moderate, even at the highest dose schedules. The combination of PALA and 5-FU at the doses and schedules used in this study did not significantly increase the activity of 5-FU. Further evaluation of these doses and schedules for activity against colorectal cancer is not warranted.**

[Cancer Treat Rep 65:747–753, 1981]

There have been few significant advances in chemotherapy for metastatic colorectal cancer since the introduction of 5-FU over two decades ago. This antimetabolite has produced objective tumor regression in 20% of the patients (1–4). When given in combination with a nitrosourea or mitomycin, the response rate could be as high as 30%–40%, although the duration of tumor response is short and its effect on patient survival is minimal (4–10). Consequently, investigation of newer chemotherapeutic agents and treatment modalities is justified.

PALA, a recently evaluated antimetabolite, inhibits de novo pyrimidine biosynthesis by blocking the activity of aspartate carbamoyl transferase (ACTase). In a phase I clinical study conducted at this institution, PALA treatment produced substantial, although transient, inhibition of tumor ACTase, with maximum effect 6 hours after therapy (11,12). In spite of this effect, the observed antitumor activity of PALA alone was modest, suggesting that blocking de novo pyrimidine biosynthesis with PALA alone was inadequate to produce tumor remission in man. However, a few minor tumor regressions were seen in patients with colorectal cancer during phase I trials (11,13). Because of the potentiating effect on the antitumor activity of 5-FU in preclinical studies (14,15) and the lack of myelosuppressive toxicity in man (11,13), a phase I–II study was initiated using PALA and 5-FU to provide additional inhibition of tumor nucleotide biosynthesis. Two treatment schedules were se-

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[3]Departments of Developmental Therapeutics (*A. Y. Bedikian, G. P. Bodey,* and *M. Valdivieso*) and Medicine (*J. R. Stroehlein, D. A. Karlin,* and *R. W. Bennetts*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Agop Y. Bedikian, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Doses by treatment schedule

| Dose level | Single dose weekly | | 5 daily doses q4wks | |
|---|---|---|---|---|
| | PALA ($g/m^2$) | 5-FU ($mg/m^2$) | PALA ($g/m^2$/day) | 5-FU ($mg/m^2$/day) |
| A | 2.5 | 600 | 1.0 | 500 |
| B* | 2.0 | 480 | 0.80 | 400 |
| C | 1.6 | 360 | 0.64 | 320 |
| D | 1.0 | 240 | 0.40 | 200 |

*Initial dose.

lected (weekly and daily × 5) to evaluate the effect of the schedule of administration of PALA and 5-FU on the antitumor activity of the combination. The results of treatment are presented here.

## PATIENTS AND METHODS

This study was conducted in a consecutive series of patients with proven metastatic colorectal cancer, who were referred to the Departments of Developmental Therapeutics and Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, between April 1979 and March 1980. Fifty-one previously untreated patients were randomized to receive single doses of PALA and 5-FU at weekly intervals (24 patients) or daily for 5 days every 4 weeks (27 patients). The initial doses of PALA were derived from our previous phase I study with this compound, where we determined recommended doses for phase II studies with PALA alone: 6 $g/m^2$ for the single-dose schedule and 1 $g/m^2$/day × 5 for the multiple-dose schedule, repeated every 2 weeks (unpublished results).

In the present study, the starting doses of both drugs in either regimen are shown as level B in table 1. The dose of 5-FU was equivalent in both treatment regimens. During a 4-week treatment period, the dose of PALA administered in the weekly schedule was equal to twice the dose administered in the 5-day schedule. In both regimens the daily dose of PALA was administered over 1 hour, while that of 5-FU was administered over 30 minutes, starting 3 hours after completion of the PALA dose. The doses of PALA and 5-FU were escalated or reduced simultaneously in both regimens to maintain a tolerable degree of hematologic (absolute neutrophil count, 750–1000/$mm^3$; platelet count, 75,000–100,000/$mm^3$) and nonhematologic toxic effects.

The antitumor responses to treatment were evaluated according to the following criteria: *complete remission*—the complete disappearance of all evidence of disease; *partial remission*—significant tumor shrinkage ≥ 50% of the sum of the products of the largest diameters of all measurable lesions; *disease stabilization*—responses less than partial remission or no change in tumor for a minimum of 8 weeks; and *disease progression*—the appearance of new lesions or a > 25% enlargement of pre-existing lesions. Prior to treatment, all patients had a complete evaluation including a history and physical examination; cbc; urinalysis; serum creatinine, alkaline phosphatase, bilirubin, SGOT, and plasma carcinoembryonic antigen (CEA) levels; and chest roentgenograms and liver scan. Ongoing analysis included weekly cbc and blood chemistry determinations, tumor measurements at 4-week intervals, and appropriate radiologic and radionuclide examinations at 2–3-month intervals. All side effects associated with treatment administration were recorded and analyzed. Prior to initiation of treatment, signed consent was obtained from each patient according to institutional policies.

Survival calculations were made for censored and uncensored data from the day of initiation of chemotherapy, by the method of Kaplan and Meier (16). A two-tailed generalized Wilcoxon test (17) was used to evaluate the statistical significance of the difference between the survival curves.

## RESULTS

Fifty-one consecutive adult patients with metastatic colorectal cancer were entered in the study. One patient died of progressive disease 10 days after initiation of treatment. The characteristics of the remaining patients are summarized in table 2. Patients receiving the two regimens were comparable in age, performance status, and frequency of liver metastasis, but there were more males receiving the weekly regimen. Antitumor responses in 50 patients are summarized in table 3. Objective tumor regression occurred in five patients receiving the weekly regimen and in six patients receiving the 5-day regimen. Partial responses were observed in four patients receiving the weekly regimen and in three receiving the 5-day regimen. The median time to response was 2 months, with a median duration of response of 3 months. Objective tumor regressions of metastases in either regimen were seen in

TABLE 2.—Patient characteristics by treatment schedule

| Characteristic | Schedule | |
|---|---|---|
| | Weekly | 5-day |
| No. of— | | |
| Patients entered | 24 | 27 |
| Early deaths | 0 | 1 |
| Patients evaluable for response | 24 | 26 |
| Males | 15 | 14 |
| Females | 9 | 13 |
| Median age in yrs (range) | 61 (30–84) | 63 (36–78) |
| Performance status (Karnofsky) | | |
| Class A | 87% | 84% |
| Class B-C | 13% | 16% |
| Liver metastasis | 64% | 69% |

TABLE 3.—Responses by treatment schedule

| Response | Schedule | |
|---|---|---|
| | Weekly | 5-day |
| No. of evaluable patients | 24 | 26 |
| No. of patients with— | | |
| Partial response | 4 | 3 |
| Disease stabilization | 15 | 12 |
| Disease progression | 5 | 11 |

the liver (seven of 32 patients), lung (five of 16), and intra-abdominal lesions (two of 18). No responses were seen in bone (three patients), lymph node (three), or subcutaneous tissue (one). The survival rates for patients treated with these regimens are shown in figures 1–3. There was no significant difference in survival duration between patients receiving the weekly regimen and those receiving the 5-day regimen (fig 1). The survival pattern by response to PALA and 5-FU combinations was similar in both regimens (figs 2 and 3).

The toxic effects of the treatment are summarized in tables 4 and 5. Toxic effects of the weekly regimen were evaluated by using four weekly treatments equivalent to a single course. Hematologic toxic effects of both regimens are compared in table 4. Despite the higher doses of PALA administered in the weekly schedule, the myelosuppression was mild in both regimens. The other toxic effects associated with the two treatment regimens are summarized in table 5. In both regimens the toxic effects were dose-dependent. Skin rash, stomatitis, and diarrhea were more frequent and severe with the weekly schedule. At the higher dose levels, generalized maculopapular skin rash was observed, and stomatitis was frequently very severe and interfered with food intake. Similarly, at the higher dose levels the diarrhea was frequently debilitating and necessitated iv fluid administration. Other toxic effects in both regimens were less frequent and required no specific treatment. In both regimens, 80% of the patients who entered at dose level B required a 50% dose reduction. Subsequently, patients were started at dose level D, but 35%–50% were able to tolerate a dose escalation to dose level C during the subsequent courses.

## DISCUSSION

Over the past 20 years, several antimetabolites have been investigated for antitumor activity against advanced gastrointestinal carcinomas. PALA is a new addition to this family of chemotherapeutic agents. It is a second-generation antimetabolite specifically designed to inhibit an early step in pyrimidine ribonucleotide de novo biosynthesis through inhibition of ACTase. In view of its unique mechanism of action, PALA, by modulation of pyrimidine nucleotide pools, could potentially enhance the antitumor activity of pyrimidine antimetabolites such

TABLE 4.—Hematologic toxicity by treatment schedule

| | Weekly schedule at dose— | | | 5-day schedule at dose— | | |
|---|---|---|---|---|---|---|
| | D | C | B | D | C | B |
| No. of courses given | 23 | 24 | 7 | 40 | 26 | 14 |
| Neutrophil count nadir | | | | | | |
| Median (cells × $10^3$/mm$^3$) | 3.6 | 3.9 | 3.8 | 4.4 | 3.5 | 4.3 |
| Range (cells × $10^3$/mm$^3$) | 1.9–9.5 | 0.6–8.7 | 2.1–4.7 | 1.9–12.5 | 0.8–8.7 | 0.1–6 |
| % courses < 1000 cells/mm$^3$ | 0 | 4 | 0 | 0 | 5 | 7 |
| Platelet count nadir | | | | | | |
| Median (cells × $10^3$/mm$^3$) | 224 | 202 | 295 | 265 | 241 | 230 |
| Range (cells × $10^3$/mm$^3$) | 50–648 | 112–534 | 175–445 | 73–604 | 72–420 | 22–392 |
| Day | 14 | 14 | 14 | 17 | 14 | 15 |
| % courses < 100,000 cells/mm$^3$ | 10 | 0 | 0 | 0 | 5 | 7 |

TABLE 5.—Nonhematologic toxic effects by treatments (%)

| | Weekly schedule at dose— | | | 5-day schedule at dose— | | |
|---|---|---|---|---|---|---|
| | D | C | B | D | C | B |
| No. of evaluable patients | 18 | 10 | 7 | 25 | 15 | 10 |
| No. of evaluable courses | 33 | 18 | 7 | 52 | 24 | 19 |
| % patients with toxic effects | 50 | 100 | 100 | 48 | 87 | 100 |
| % courses with: Toxic effects | 45 | 67 | 100 | 31 | 79 | 79 |
| Skin rash | 9 | 33 (10)* | 57 (29) | 4 (2) | 14 (6) | 35 (20) |
| Stomatitis | 12 | 28 (6) | 57 (43) | 16 (3) | 54 (17) | 30 (15) |
| Diarrhea | 6 (3) | 33 (5) | 43 (43) | 8 (2) | 17 (6) | 35 (20) |
| Nausea/vomiting | 21 | 16 | 29 | 11 | 23 | 15 |
| Abdominal cramps | 3 | 5 | 14 | 3 | — | 15 |
| Fever of unknown origin | — | — | 14 | — | 9 | 10 |
| Paresthesias | — | — | 14 | — | 3 | 10 |
| Anorexia | 9 | 16 | — | 3 | 6 | 10 |
| Conjunctivitis/keratitis | 3 | — | — | 2 | 3 | — |

*Nos. in parentheses = % of courses resulting in severe toxicity.

FIGURE 1.—Survival by treatment schedule.

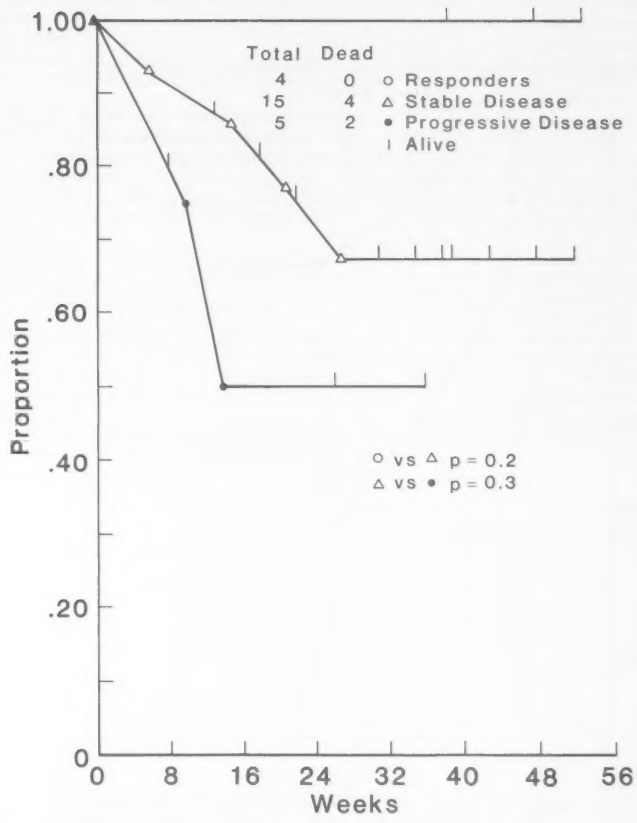


FIGURE 2.—Survival with weekly schedule, by response.

as 5-FU. Preclinical studies that were published only recently indicated that a PALA and 5-FU combination was synergistic at a wide range of PALA and 5-FU concentrations (18). This synergism was considered to result from enhancement of incorporation of 5-FU into tumor cell RNA in the presence of PALA (18–20). Also, prolonged exposure of tumor cells to the PALA and 5-FU combination resulted in a 1.4–2.3-fold increase in incorporation of 5-FU into RNA compared to that observed with 5-FU alone (15,18).

Unfortunately, the results of our study indicate that the antitumor activity of the PALA and 5-FU combination against human large bowel cancer presents no marked advantage over that seen with 5-FU alone. The administration of PALA 3 hours before 5-FU in either schedule did not increase the antitumor activity of 5-FU so as to produce a significant increase in response rates and survival durations. Also, the antitumor activity of the PALA and 5-FU combination was not influenced by the dose of PALA administered. The higher dose of PALA administered with the weekly schedule was associated with higher incidences and more severe episodes of mucocutaneous and gastrointestinal toxic effects than those seen with the 5-day schedule. Similarly, the 5-day schedule did not result in higher response rates or longer survival durations than those seen in the weekly schedule.

The low response rates seen in both treatment schedules in this study cannot be completely attributed to the lower dose of 5-FU administered at the tolerable dose level for the PALA and 5-FU combination. Although the simultaneous dose adjustment required by the design of the study resulted in a reduction in the 5-FU dose to one half of the

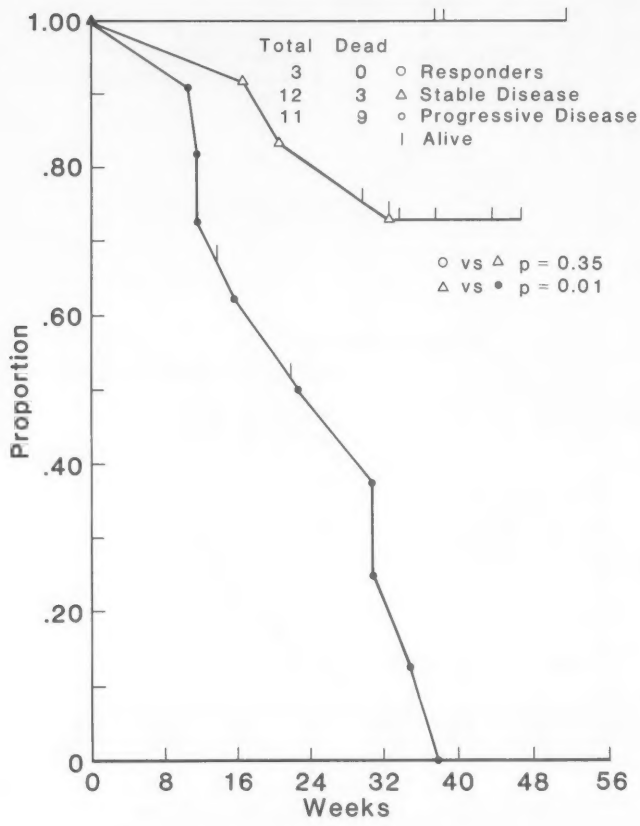


FIGURE 3.—Survival with 5-day schedule, by response.

originally intended dose, mainly because of the severe mucocutaneous and gastrointestinal toxic effects of the PALA and 5-FU combination, the response rate in patients treated with the initial high doses of PALA and 5-FU was not any higher than that obtained at the reduced doses. Pharmacology studies of PALA and 5-FU currently under way may elucidate the metabolic interaction resulting from the administration of these drugs by the schedule used in this study. This combination of PALA and 5-FU at the doses and schedules used in this study did not seem to increase the activity of 5-FU significantly. Further evaluation of these doses and schedules for activity against colorectal cancer is not recommended.

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# Phase I Trial of Pentamethylmelamine: A Clinical and Pharmacologic Study[1]

**Daniel C. Ihde,* John S. Dutcher, Robert C. Young, Rosemarie S. Cordes, Audrey L. Barlock, Susan M. Hubbard, Roy B. Jones, and Michael R. Boyd[2]**

## SUMMARY

**Pentamethylmelamine (PMM) is a water-soluble monodemethylated derivative of hexamethylmelamine and has a similar spectrum of activity against murine tumors. Unlike hexamethylmelamine, it is suitable for parenteral administration. We conducted a phase I trial of PMM given as a weekly 1-hour iv infusion to 34 extensively pretreated patients with advanced solid tumors. Eight dose levels ranging from 80 to 1500 mg/m² were studied. A median of three infusions (mean, 4.2; range, 1–24) were given to each patient. Severe nausea and vomiting was dose-limiting at 1500 mg/m²; it was unresponsive to antiemetics and persisted up to 48 hours. Mild to moderately depressed levels of consciousness were seen in one third of the patients at dose levels of ⩾ 750 mg/m². Consistent dose-related myelosuppression was not observed. Hepatocellular toxicity manifested by elevated serum transaminases occurred sporadically, usually in patients with liver metastases, but could not be unequivocally attributed to the drug. No complete or partial tumor responses were noted. At each dose level, the pharmacokinetics of PMM disappearance from plasma were studied in one to three patients with a gas chromatograph-mass spectrometer assay which exclusively measured the unmetabolized drug. The data obtained were consistent with a two-compartment model of drug distribution, with a mean dose-independent terminal half-life of 143 minutes (range, 43–370). Peak drug levels were directly proportional to dose. The relative lack of myelotoxicity would make PMM an attractive candidate for addition to combination regimens if antitumor activity at tolerable doses could be documented. On this schedule, the recommended dose is 1000 mg/m² for phase II trials.**

[Cancer Treat Rep 65:755–762, 1981]

Hexamethylmelamine (HMM), an *s*-triazine compound structurally related to the alkylating agent triethylenemelamine, is an antitumor agent with activity against several human cancers, including carcinomas of the ovary and lung and malignant lymphomas (1). Because of its poor aqueous solubility, HMM is suitable only for oral administration. It has been utilized in a continuous daily schedule or in intermittent 2-week courses in which gastrointestinal toxicity is most often dose-limiting (1). Since plasma levels of orally administered HMM are unpredictable and unrelated to dose in man (2), analogs of HMM that could be given parenterally were sought.

The monodemethylated HMM derivative pentamethylmelamine (PMM) is 23.7 times as water-soluble as the parent compound and can be administered iv (3). Although the therapeutic index of HMM

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[2]National Cancer Institute-Veterans Administration Medical Oncology Branch (Washington, DC) and Medicine and Clinical Pharmacology Branches (Bethesda, MD), Division of Cancer Treatment, National Cancer Institute.

**Reprint requests to:* Dr. Daniel C. Ihde, National Cancer Institute-Navy Medical Oncology Branch, National Naval Medical Center, Bethesda, MD 20014.

against the murine ADJ/PC6 plasma cell tumor is much superior to that of PMM (4), the two drugs have approximately similar antitumor effects at equitoxic doses in the Sarcoma 180 and Lewis lung carcinoma murine systems (5). HMM and PMM were equally active at identical doses when tested against the human lung cancer xenograft P246 (6). Thus, PMM appeared to be an excellent candidate for introduction into clinical trials.

Large animal toxicology of PMM was performed in dogs and monkeys,[3] with single-dose, daily × 5, and daily × 10 schedules being studied in the former and daily × 5 schedules being studied in the latter. The major toxic effects observed were seizures, hyperexcitability, ataxia, somnolence, incontinence, diarrhea, and melena. They occurred during or immediately following iv injection. Neurologic manifestations could be greatly reduced by decreasing the rate of drug administration. Anorexia and weight loss were also noted. Pathologically, the major toxic effects of PMM included skin necrosis and inflammation with drug infiltration, hemorrhage and decreased cellularity in lymphatic tissues, crystal deposition and glomerular atrophy in the kidney, and testicular degeneration. Effects on the bone marrow and liver were minimal. Transient leukopenia, thrombocytopenia, and elevated transaminases occurred only at sublethal doses. The toxic dose low in dogs was 250 mg/m² for single and daily × 5 administration and 125 mg/m² on a daily × 10 schedule.

In October 1978, we initiated a phase I trial of weekly administration of PMM in patients with advanced solid tumors. Because of the dependence of neurologic toxicity on the rate of administration in preclinical studies, the drug was given as a 1-hour iv infusion. In addition to determining the maximum dose of PMM which would produce tolerable, predictable, and reversible toxicity, a further objective of this study was the investigation of the pharmacokinetics of the drug, utilizing our previously described assay (7).

## PATIENTS AND METHODS

### Clinical Studies

PMM was administered to 34 patients with nonhematologic solid tumors; their pretreatment characteristics are listed in table 1. The majority of the patients were fully ambulatory, and almost all had been extensively pretreated with chemotherapy. All patients had unresectable cancer not amenable to curative irradiation; 32 had developed tumor progression while receiving one or more standard chemotherapeutic regimens, and two had malignancies for which no effective drugs are known. Other eligibility criteria included a life expectancy of at least 8 weeks, recovery from toxic effects induced by previous therapy, no major impairment of mental status, ability and willingness to give informed consent, and adequate hematologic, hepatic, and renal functions (wbc count > 3500/μl, platelet count > 80,000/μl, serum bilirubin and creatinine < 2.0 mg/dl, SGOT < 80 units/ml, and ≤ 1+ proteinuria by dipstick).

TABLE 1.—Characteristics of 34 patients given PMM

| Characteristic | No. of patients |
|---|---|
| Median age in yrs (range): 56 (23–72) | |
| Sex | |
| Males | 24 |
| Females | 10 |
| Prior therapy | |
| Chemotherapy only | 13 |
| Radiotherapy only | 1 |
| Both | 19 |
| Neither | 1 |
| Performance status* | |
| 0–1 | 19 |
| 2 | 10 |
| 3 | 5 |
| 4 | 0 |
| Primary tumor site | |
| Lung | 9† |
| Stomach | 1 |
| Colon | 2 |
| Prostate | 1 |
| Kidney | 1 |
| Unknown primary | 3 |
| Breast | 3 |
| Ovary | 3 |
| Melanoma | 5 |
| Head and neck | 3 |
| Soft tissue sarcoma | 2 |
| Osteosarcoma | 1 |

*Eastern Cooperative Oncology Group scale: 0, 1 = fully ambulatory; 2 = ambulatory > 50% of day; 3 = ambulatory < 50% of day; 4 = totally bedridden.

†Includes 4 patients with small cell carcinoma.

PMM (as the hydrochloride) was supplied by the Investigational Drug Branch, National Cancer Institute, Bethesda, MD, in the form of a white lyophilized powder. Each vial contained 500 mg of the drug, 300 mg of mannitol, and sufficient sodium hydroxide to adjust the pH to 4.5–5.5 when reconstituted. PMM was reconstituted with 9.5 ml of sterile water and was diluted in 100–250 ml of 5% dextrose in water or normal saline for iv administration. The

[3]Clinical brochure: pentamethylmelamine, NSC 118742. Prepared by the National Cancer Institute, Bethesda, MD, Sept 1978.

drug was given as a weekly 1-hour iv infusion at constant infusion rate. At the end of the trial four patients received the drug as a 6-hour infusion in a volume of 400–500 ml. Two weekly infusions were planned for all patients. Treatment was continued thereafter until the development of tumor progression, unacceptable toxicity, or patient refusal to accept further therapy.

The initial dose of PMM was 80 mg/m², approximately one third the lowest toxic single dose in dogs. Eight dose levels from 80 to 1500 mg/m² were studied. Doses were not escalated in individual patients.

Immediate gastrointestinal and neurologic toxic effects, for which all patients were considered evaluable, were monitored by direct observation. During therapy, cbcs, automated blood chemistries, and urinalyses were performed weekly. Three weeks' observation was required for a patient to be considered evaluable for hematologic or biochemical toxicity, unless toxicity developed more quickly.

Evaluable tumors were present in 33 of 34 patients. Evaluation of antitumor effects required 4 weeks' observation, or the more rapid development of tumor progression. Complete and partial responses were defined as the disappearance or 50% reduction of all clinical evidence of tumor, persisting for at least 4 weeks; minor response was defined as a lesser degree of tumor regression; stable disease was defined as the lack of tumor progression for at least 8 weeks; and progressive disease was defined as an increase of ≥ 25% in evaluable tumors or development of new tumors.

### Pharmacologic Studies

Pharmacokinetic analyses in plasma were performed after 19 infusions in 15 patients. A mean of nine plasma samples (range, six to 13) was obtained for each analysis. In two patients, single plasma PMM levels were determined at the end of a 6-hour infusion. Drug levels were assayed in the urine of eight patients and in the cerebrospinal fluid (CSF) of one. At timed intervals after iv infusion, blood samples were collected in heparinized Vacutainer tubes (Fisher Scientific Co, Pittsburgh, PA) through a heparin lock distant from the infusion site. After centrifugation at 1000 × *g* for 20 minutes, plasma was separated and stored at –78°C until analysis. CSF and 24-hour urine samples were stored in a similar fashion.

Plasma PMM concentrations after doses of 80, 160, and 250 mg/m² and all urinary PMM concentrations were determined using the gas chromatograph-mass spectrometer (GC-MS) method previously reported (7). Samples obtained from patients receiving higher doses required modification of reagent volumes. A mixture of 1 ml of plasma, 1 ml of internal standard solution (2 μg/ml of aqueous solution of deuterium-labeled PMM hydrochloride), 0.5 ml of 1 N NaOH, and 5 ml of ethyl acetate was shaken for 30 seconds. After centrifugation at 1000 × *g* for 5 minutes, the organic solution was transferred to a 5-ml Reacti-Vial (Pierce Chemical Co, Rockford, IL) and was evaporated under a stream of nitrogen. The residue was dissolved in 25 μl of ethyl acetate and 5 μl was injected into the GC-MS. Samples of CSF were analyzed similarly. The highest doses studied (1000 and 1500 mg/m²) required further modification of extraction volumes and internal standard concentration (0.1 ml of plasma, 0.1 ml internal standard of 20 μg/ml, 0.05 ml of NaOH, and 2 ml of ethyl acetate).

Plasma PMM concentrations were fit to two- or three-compartment models using the MLAB curve fitting program (8). Intercepts at the end of infusion and disposition rate coefficients were then used to calculate half-lives, clearances, volumes of distribution, and rate coefficients (9).

## RESULTS

At least three patients were treated at each of the eight dose levels of PMM. A total of 142 infusions (median, three per patient; mean, 4.2; range, one to 24) were administered. The number of patients treated and the median number of infusions per patient at each dose level are listed in table 2.

### Toxicity

The major toxic effects of PMM given in this schedule were gastrointestinal, with severe, protracted, dose-related nausea and vomiting that was

TABLE 2.—Dose escalation and No. of infusions given at each dose

| Dose (mg/m²) | No. of patients | Infusions/patient | |
|---|---|---|---|
| | | Median | Range |
| 80 | 3 | 4 | 4–5 |
| 160 | 3 | 5 | 2–6 |
| 250 | 4 | 5 | 2–24 |
| 400 | 3 | 3 | 3–7 |
| 550 | 3 | 4 | 2–9 |
| 750 | 3 | 3 | 1–4 |
| 1000 | 6 | 3 | 2–9 |
| 1500 | 9* | 2 | 1–5 |
| Totals | 34 | 3 | 1–24 |

*5 patients received 1-hr infusions only; 3 received 6-hr infusions only; and 1 received both.

TABLE 3.—Gastrointestinal and neurologic toxic effects of PMM

| Dose ($mg/m^2$) | No. of evaluable patients | Nausea and vomiting* | Impaired level of consciousness† |
|---|---|---|---|
| 80 | 3 | 3/0/0/0‡ | 3/0/0/0 |
| 160 | 3 | 2/1/0/0 | 2/1/0/0 |
| 250 | 4 | 2/2/0/0 | 4/0/0/0 |
| 400 | 3 | 1/2/0/0 | 3/0/0/0 |
| 550 | 3 | 1/0/2/0 | 3/0/0/0 |
| 750 | 3 | 1/1/1/0 | 2/0/1/0 |
| 1000 | 6 | 1/1/1/3 | 4/2/0/0 |
| 1500 | 9 | 0/1/1/7 | 6/2/1/0 |

*Toxicity grades: mild = nausea (< 4 emeses); moderate = emesis for < 12 hrs; severe = emesis for 12–24+ hrs.

†Toxicity grades: mild = lethargy; moderate = somnolence; severe = coma.

‡No. of patients with none/mild/moderate/severe toxic effects.

TABLE 4.—Hematologic and hepatocellular toxic effects of PMM

| Dose ($mg/m^2$) | No. of evaluable patients | Leukopenia* | Thrombocytopenia* | Hepatocellular toxicity† |
|---|---|---|---|---|
| 80 | 3 | 3/0/0/0‡ | 2/1/0/0 | 2/0/0/1 |
| 160 | 2 | 2/0/0/0 | 2/0/0/0 | 1/0/1/0 |
| 250 | 3 | 1/2/0/0 | 3/0/0/0 | 2/0/0/1 |
| 400 | 3 | 3/0/0/0 | 3/0/0/0 | 3/0/0/0 |
| 550 | 3 | 3/0/0/0 | 2/1/0/0 | 3/0/0/0 |
| 750 | 3 | 3/0/0/0 | 3/0/0/0 | 1/2/0/0 |
| 1000 | 5 | 4/0/1/0 | 4/1/0/0 | 3/2/0/0 |
| 1500 | 7 | 5/1/1/0 | 6/1/0/0 | 5/1/1/0 |

*Toxicity grades ($cells/mm^3$): mild = wbcs, 2500–3900, and platelets, 50,000–99,000; moderate = wbcs, 1000–2400, and platelets, 25,000–49,000; severe = wbcs < 1000, and platelets < 25,000.

†Toxicity grades (SGOT, units/ml): mild < 100; moderate = 101–200; severe > 200.

‡No. of patients with none/mild/moderate/severe toxic effects.

unresponsive to usual antiemetics and proved to be dose-limiting at a dose of 1500 $mg/m^2$. Emesis was first noted after a dose of 160 $mg/m^2$ (table 3), and in some patients became moderate in degree at 550 $mg/m^2$ and severe at 1000 $mg/m^2$. Vomiting persisted for up to 48 hours in a few patients treated at a dose of 1500 $mg/m^2$. When severe, vomiting was accompanied by marked diaphoresis in four patients and by reversible hypotension in one. All six patients given a 1-hour infusion at a dose of 1500 $mg/m^2$ had severe emesis. When the infusion was prolonged to 6 hours in an effort to reduce toxicity, vomiting was mild in one patient, moderate in one, and severe in two.

Nausea and vomiting led to refusal to continue PMM treatment in five patients. With a 1-hour infusion, the maximum tolerated dose in our patients was 1000 $mg/m^2$. Other gastrointestinal effects, including diarrhea in one patient and hiccoughs in two, were only questionably related to PMM. Many patients reported anorexia during therapy, and weight loss > 3 kg (range, 3.5–13.5) was recorded for six patients.

Neurologic toxic effects of PMM, which were also dose-related, are listed in table 3. Approximately one third of the patients treated at doses of ≥ 750 $mg/m^2$ developed transient depressed levels of consciousness beginning during or shortly after the infusion prior to the administration of any antiemetic drugs. Somnolence persisted for 2 and 12 hours in the two most severely affected patients.

Twenty-nine patients were evaluable for hematologic and biochemical toxicity (table 4). Hematologic toxicity was observed in only nine patients, was mild to moderate in all, was reversible with discontinuation or occasionally even with uninterrupted use of the drug, and was not clearly dose-related.

Possible hepatocellular toxicity of PMM was noted in nine patients, seven of whom were known to have liver metastases (table 4). This toxicity was manifested by elevations in transaminase (SGOT and SGPT) but not by increases in serum bilirubin. It was reversible with continued drug treatment in three patients and with cessation of PMM in one.

Other sporadic abnormalities seen during the study

were most likely unrelated to administration of PMM. They included congestive heart failure, hypercalcemia, paresthesias, and atrial fibrillation with mild hypotension during infusion in single patients; in the latter instance, the patient was re-treated without incident. Two patients developed elevated serum creatinine values which later normalized in one. No abnormalities in blood glucose, serum electrolytes, or urinalysis were observed.

## Antitumor Effects

No complete or partial responses to PMM were noted. Two minor responses, consisting of a marked diminution in malignant ascites in a patient with small cell lung carcinoma and a < 50% shrinkage of a subcutaneous nodule in a patient with colon cancer, occurred. Three patients had stable disease for 9, 10, and 32 weeks. Progressive disease during therapy was documented in 20 patients. The remaining nine patients were not evaluable for response because of refusal to continue treatment (five patients), early death (three), or lack of evaluable tumor (one).

## Pharmacologic Studies

Plasma elimination of PMM was studied in at least one patient at every dose. Samples were initially drawn during and after infusion. Plasma levels increased regularly until the infusion was stopped and then rapidly declined. In most later studies, samples were obtained only after infusion. A plasma elimination curve (plasma concentrations vs time and the corresponding computer fit) for one representative patient at each dose level is shown in figure 1. In general, the values were fit best by a two-compartment model with elimination from the central

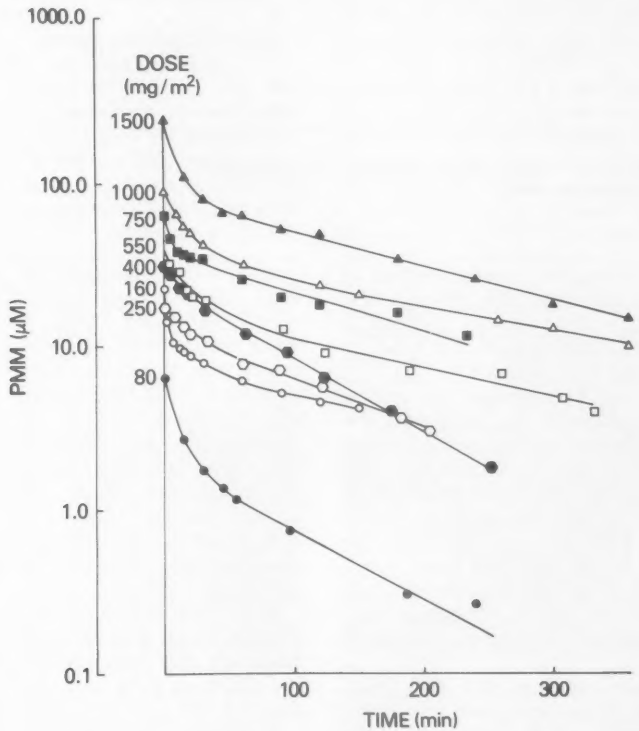


FIGURE 1.—Parent drug concentration vs time curves at PMM doses from 80 to 1500 mg/m² administered as 1-hr infusions; determined by direct measurement (various symbols) and computer fit (—). Each curve = typical patient at the stated dose.

TABLE 5.—Kinetic parameters of PMM administered as a 1-hr iv infusion

| Dose ($mg/m^2$) | Patient No. | Infusion No. | Peak level ($\mu M$) | Elimination half-life (mins) | Plasma clearance (ml/min/kg) |
|---|---|---|---|---|---|
| 80 | 1 | 1 | ND* | 97 | 27.5 |
| | | 2 | 2 | 115 | 28.7 |
| | 2 | 1 | ND | 290 | 14.3 |
| | | 2 | 6 | 164 | 12.1 |
| | 3 | 1 | 6 | 70 | 13.5 |
| | | 3 | 5 | 52 | NC† |
| 160 | 4 | 4 | 23 | 80 | NC |
| | 5‡ | 3 | 18 | 348 | NC |
| 250 | 6‡ | 1 | 19 | 56 | NC |
| | 7 | 1 | 22 | 84 | 10.9 |
| | | 2 | 18 | 100 | 11.7 |
| | 8‡ | 1 | ND | 370 | 9.6 |
| 400 | 9 | 1 | 34 | 43 | 13.7 |
| | 10 | 1 | 49 | 74 | 8.8 |
| 550 | 11 | 1 | 37 | 176 | 10.3 |
| 750 | 12 | 1 | 65 | 123 | 8.2 |
| 1000 | 13‡ | 1 | 95 | 215 | 7.1 |
| 1500 | 14 | 1 | 154 | 114 | 6.7 |
| | 15 | 1 | 245 | 143 | 5.5 |

*ND = not done.
†NC = not calculated due to lack of data in distribution phase of curve.
‡Fit using a 3-compartment model.

compartment. The means and standard errors of the coefficients characterizing the two-compartment model were: $k_{10} = (3.2 \pm 0.6) \times 10^{-2}\ min^{-1}$, $k_{12} = (4.9 \pm 1.2) \times 10^{-2}\ min^{-1}$, $k_{21} = (2.2 \pm 0.5) \times 10^{-2}\ min^{-1}$, $V_1 = 0.63 \pm 0.14$ l/kg, and $V_2 = 0.77 \pm 0.17$ l/kg (where $V_1$ and $V_2$ represent the central and peripheral compartments, $k_{10}$ represents the elimination rate coefficient, and $k_{12}$ and $k_{21}$ represent the forward and reverse distribution rate coefficients). In four patients the elimination was triphasic and was fit best with a three-compartment model.

Table 5 shows the PMM doses administered, the corresponding peak levels (obtained from 0 to 5 minutes after the end of the infusion), the terminal-phase half-lives, and the plasma clearances (normalized to body weight) for all of the patients studied.

Peak levels increased regularly with dose; least-squares analysis of the dose versus peak level (table 5) gave a correlation of $r^2 = 0.89$. Terminal half-life (mean ± standard error, 143 ± 22 minutes; range, 43–370) was unrelated to the dose of PMM administered. Plasma clearance values, however, did appear somewhat dose-dependent. The slope determined by least-squares analysis of the dose versus clearance curve was different from zero ($P = 0.07$, two-tailed t test). Although patients with major compromise of hepatic function were excluded from this trial, there were no apparent alterations in PMM pharmacokinetics in seven patients with increased serum levels of SGOT or alkaline phosphatase. One patient became markedly somnolent after approximately one half of a dose of 750 $mg/m^2$ had been infused. Drug administration was stopped and simultaneous CSF and blood samples were obtained approximately 40 minutes later. PMM concentrations in these samples were 42 $\mu M$ (plasma) and 10 $\mu M$ (CSF).

In two patients who were given PMM as a 6-hour infusion, single plasma levels were determined immediately after cessation of infusion. These levels of 5 and 6 $\mu M$ were only 2%–4% of the peak levels attained with 1-hour infusions at the identical dose.

Analyses of aliquots from 24-hour urine collections indicated that at all dose levels an average of < 0.05% of the PMM dose was excreted unchanged. The largest fraction of unmetabolized drug in the urine of any patient was 0.27% of the administered

PMM dose. In patient 12, from whom urine was collected in 8-hour fractions, 0.24% of the administered dose was recovered in the urine during 0–8 hours, 0.03% during 8–16 hours, and a negligible percent during 16–24 hours.

## DISCUSSION

The principal clinical toxic effects of HMM are gastrointestinal, neurologic, and hematologic (1). Since PMM is the initial demethylated metabolite of HMM in man (10,11), the dose-limiting nausea and vomiting and dose-related decreased levels of consciousness which we encountered in this phase I trial of PMM were not unexpected. At higher PMM doses, emesis was severe and protracted and responded poorly, if at all, to antiemetics. Prolonging infusion time in a few patients considerably reduced plasma levels of the drug at the end of infusion and may have modestly reduced clinical toxicity, but treatment of more patients would be required to substantiate this point.

The central nervous system toxicity observed in this study was definite but mild in degree at the maximum tolerated dose of 1000 mg/m². The predominance of a depressed sensorium over any other neurologic side effects differs from the results seen with administration of HMM (1). This might be explained by the much higher plasma levels which are achieved with iv PMM compared to oral HMM given in standard doses (2) and the relatively high CSF to plasma ratio of PMM concentrations which we were able to document in one patient. The peak concentration of radiolabeled PMM in mouse brain is threefold greater than that attained after a comparable dose of labeled HMM (12).

Myelosuppression in our patients was of little clinical significance, since it was mild to moderate in degree, sporadic, and unrelated to dose in these heavily pretreated patients. Since the median number of infusions administered was only three, however, it is possible that more predictable hematologic toxicity might have developed with more protracted therapy, as was true with long-term administration of low doses of HMM (1). In another recently concluded phase I trial of relatively short-term administration of PMM (13), marked but reversible myelosuppression occurred only at the extremely high dose of 1200 mg/m² given for 10 consecutive days. This dose schedule was intolerable due to severe nausea and vomiting and central nervous system effects.

No significant objective tumor responses were observed in the present phase I trial, and it is currently unknown whether clinically tolerable doses of PMM possess antitumor effects. With HMM, response rates were significantly increased when leukopenia or thrombocytopenia was produced in one broad phase II trial (14). However, this was not true in other studies which reported fewer responses (15,16), and in one randomized trial doubling the dose of HMM did not improve the response rate (16). Thus, the absence of significant hematologic toxicity at the maximum tolerated doses of PMM in this weekly schedule does not necessarily preclude antitumor effects.

Possible hepatocellular toxicity of PMM manifested in our patients by elevated transaminase was not dose-related and usually occurred in patients with liver metastases. These abnormalities in liver function tests are thus difficult to firmly ascribe to the drug.

Our pharmacokinetic analyses indicate that plasma elimination of PMM is best described by a two-compartment model, with average half-lives of 13 ± 4 and 125 ± 23 minutes for the distribution and elimination phases, respectively. In a few patients, the elimination phase was more consistent with a three-compartment model, with an average terminal half-life of 247 ± 3 minutes. The apparent differences between half-lives for the two- versus three-compartment models may be somewhat exaggerated, however, since the standard errors for two of the four individual three-compartment fits were much greater than with the two-compartment fits.

Ames et al (17) obtained similar pharmacologic data at lower PMM doses in a small number of patients. They also demonstrated that the elimination of PMM from human plasma was accompanied by the detection of multiple successively demethylated metabolites of the parent compound. This rapid metabolism of PMM, presumably in the liver, is supported by our finding that virtually no intact drug is excreted in the urine.

Severe nausea and vomiting limits the maximum tolerated dose of PMM given as a weekly 1-hour iv infusion to approximately 1000 mg/m². If antineoplastic efficacy could be demonstrated with this or some other tolerable schedule, iv PMM could be an attractive alternative to HMM for incorporation into combination chemotherapy programs since predictable blood levels could be attained with little or no added myelosuppression. The mechanism of action of PMM and HMM is not fully understood, but both drugs require metabolic activation to produce cytotoxic effects in vitro (4,18). In the system studied by Meltz and Winters (18), a 3-hour exposure to 100 μg/ml of PMM in the presence of a liver microsomal preparation substantially retarded the growth of a human malignant cell line. This concentration is approximately fourfold higher than the 100 μM peak drug levels we observed after a 1-hour infusion of

1000 mg/m$^2$. Unfortunately, lower concentrations of PMM were not tested by Meltz and Winters (18), but further investigation using this or other similar in vitro techniques might aid in establishing that clinically tolerable PMM plasma concentrations have tumoricidal effects. Such data could encourage the more rapid entry of PMM into phase II trials. A direct comparison of PMM with HMM in a human tumor in which HMM possesses considerable antitumor activity could be another rational way to investigate whether PMM may eventually prove to be useful in cancer chemotherapy.

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# Influence of Estrogen Receptor Status on Response to Combination Chemotherapy for Recurrent Breast Cancer[1]

Joanne Mortimer,* Ronald Reimer, Richard Greenstreet, Carl Groppe, and Ronald Bukowski[2,3]

## SUMMARY

**To determine the influence of cytoplasmic estrogen receptor (ER) status on response to chemotherapy among patients with recurrent breast cancer amenable to biopsy, we studied 65 patients who had ER determinations performed on metastatic lesions. The response to combination chemotherapy and duration of survival were determined in 49 of these patients who received cytotoxic chemotherapy as their sole modality of treatment. Compared to patients with ER levels < 3 femtomols/mg, patients with an ER concentration ≥ 3 femtomols/mg had a higher response rate (57% vs 21%, $P < 0.05$) and a longer survival (35 vs 13 months, $P < 0.0003$). Some of the increased survival of the ER-positive patients was attributable to response to subsequent hormonal manipulations. These differences between ER-positive and ER-negative patients were lessened by redefining ER-positive as ≥ 10 femtomols/mg. Nevertheless, at our institution patients with ER-positive metastatic breast cancer have a higher response rate to chemotherapy and survive longer than their ER-negative counterparts.**

[Cancer Treat Rep 65:763–766, 1981]

Approximately one half of the nearly 100,000 patients developing breast cancer annually will have recurrences (1). While most of these patients eventually die from their illness, long-term palliation is often possible with endocrine manipulations, cytotoxic chemotherapy, and/or radiation therapy.

The discovery of the estrogen receptor (ER) in the cytoplasm of some breast cancer cells has aided the clinician in selecting patients for hormonal manipulations (2). Recently, several investigators have questioned the influence of ER status on other variables such as survival after mastectomy and response to chemotherapy for metastatic disease. While most series indicate that patients bearing ER-positive primary breast cancers have a better prognosis than those lacking ER (3,4), studies of the effect of ER status on response to chemotherapy have yielded conflicting results (5,6). Carter (7) has suggested that some of these differences may be attributable to different definitions of "ER-positive" or to different laboratory techniques. In addition, many of the ER determinations were from the primary tumor rather than a metastatic lesion. With this in mind, we retrospectively reviewed patients with breast cancer whose metastases had been analyzed for ER at our institution.

## MATERIALS AND METHODS

A single observer (J. Mortimer) reviewed the records of 65 consecutive patients whose metastatic breast cancers had been evaluated and treated at the Cleveland Clinic Foundation from 1975 to 1977. All patients had had ER determinations performed on pathologically proven metastatic lesions prior to the beginning of therapy. Treatment decisions during these years had been made independent of knowledge of ER status. A subset of 49 patients

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[2]Departments of Hematology and Biostatistics, Cleveland Clinic Foundation, OH.

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*Reprint requests to: Dr. Joanne Mortimer, Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44106.

had received only chemotherapy as their first systemic therapy for metastatic disease. These 49 patients form the basis for this report; the other 16 patients had received hormonal therapy alone or in combination with chemotherapy and were thus excluded.

ER analysis was performed by the charcoal gradient method (8). Several specimens were also measured by sucrose gradient and in a reference laboratory. Correlation was excellent. Response to chemotherapy and survival time following recurrence were determined for all patients. ER-positive versus ER-negative patients were compared twice, once each using a definition of ER-positive as ≥ 3 femtomols/mg and as ≥ 10 femtomols/mg.

Chemotherapy for recurrent disease consisted of a combination of cyclophosphamide, vincristine, methotrexate, 5-FU, and prednisone, as previously described (10). Complete response (CR), partial response (PR), stable disease, and progressive disease were defined in a standard manner (11).

Survival was calculated by the Kaplan-Meier method (12). Statistical comparison of survival curves was done by the Gehan-Breslow method (13). Comparison of percents was done by the chi-square technique (14).

## RESULTS

Table 1 lists the pretreatment characteristics of the 49 patients. No patient had received prior adjuvant systemic therapy. ER-positive and ER-negative groups are comparable with respect to menopausal status, disease-free interval, previous radiation therapy, and sites of metastasis. The only significant difference between the two groups was a higher frequency of hepatic metastases among ER-positive patients.

As summarized in table 2, 30 patients had ER concentrations ≥ 3 femtomols/mg. Responses to chemotherapy were observed in 17 patients (CR + PR = 57%). Nine patients showed a CR, with a median duration of response of 18 months and a median survival of 52 months. Dominant sites of metastases in this group included bone (two patients), lung (three), lymph node (two), and cutaneous (two). Of a total of 15 metastatic sites, one was hepatic. PRs were seen in eight patients, with a median duration of response of 21 months and a median survival of 46 months. Dominant sites of metastases were bone (two patients), lung (two), lymph node (two), cutaneous (one), and hepatic (one). Three of the total 15 metastatic sites were hepatic. An additional seven patients exhibited stable disease after chemotherapy. Five of these patients were postmenopausal with primarily osseous metastases. Only six of the 30 ER-positive patients (20%) had disease progression.

TABLE 1.—Pretreatment characteristics of 49 patients with metastatic breast cancer

| Characteristic | ER < 3 femtomols/mg | ER ≥ 3 femtomols/mg |
|---|---|---|
| | *No. of patients* | |
| Total evaluable | 19 | 30 |
| Premenopausal | 7 (37%) | 8 (27%) |
| Postmenopausal | 9 (49%) | 18 (60%) |
| Perimenopausal | 3 (15%) | 4 (13%) |
| Prior radiation therapy | 6 (32%) | 6 (20%) |
| Median disease-free interval (mos) | 30 | 23 |
| | *No. of metastases* | |
| Sites of metastases | | |
| Chest wall | 9 | 13 |
| Lymph node | 7 | 12 |
| Lung | 5 | 7 |
| Liver | 2* | 11* |
| Bone | 5 | 12 |

*$P < 0.05$.

The median duration of response to chemotherapy was 16 months in these 30 patients. Thirteen patients demonstrated relapse or second recurrence of their disease and received subsequent hormonal therapy with estrogens, androgens, or tamoxifen. Six patients demonstrated a response to therapy while four had stabilization of their tumor. Overall median survival time from diagnosis of recurrent disease was 35 months.

Nineteen patients had ER concentrations < 3 femtomols/mg. Only four of these patients responded to chemotherapy (CR + PR = 21%). Median duration of response was 8 months. The one patient who demonstrated a CR had predominantly cutaneous metastases. Three patients had PRs. Dominant sites of metastases were lung, lymph node, and liver. Of five total metastatic sites, one was hepatic. The median survival time after recurrence for all 19 patients was 13 months.

The differences in response rate (57% when ER ≥ 3 femtomols/mg vs 20% when ER < 3 femtomols/mg) and survival time (35 months when ER ≥ 3 femtomols/mg vs 13 months when ER < 3 femtomols/mg) are significant at $P < 0.05$ and $P < 0.0003$, respectively.

When the definition of ER-positive is changed to ≥ 10 femtomols/mg, the assay is less predictive of response to chemotherapy, with 54% of ER-positive patients and 32% of ER-negative patients responding ($P = 0.2$). Overall survival time from first recurrence, however, is still significantly longer in the ER-positive group (35 vs 15 months, $P < 0.005$).

TABLE 2.—Response to chemotherapy as a function of ER concentration

| | No. of patients with ER levels (femtomols/mg) of— | | | |
|---|---|---|---|---|
| | < 3 | ≥ 3 | < 10 | ≥ 10 |
| Total evaluable | 19 | 30 | 25 | 24 |
| CR + PR | 4 (21%) | 17 (57%) | 8 (32%) | 13 (54%) |
| Stable disease | 6 (32%) | 7 (23%) | 7 (28%) | 6 (25%) |
| No response | 9 (47%) | 6 (20%) | 10 (40%) | 5 (21%) |

## DISCUSSION

Previous surveys of the influence of ER status on response to chemotherapy in metastatic breast cancer have yielded conflicting results (5–7). Some centers have demonstrated that patients lacking ER have a higher response rate and longer survival after chemotherapy for metastatic disease, while others have observed opposite results.

Several explanations have been advanced for this discrepancy, including methods in determining ER content, variations in systemic chemotherapy selected, differences in the definition of ER positivity, and utilization of ER data from the primary tumor as opposed to the metastatic lesion. This latter point, in particular, appears relevant in that 20% of patients may experience a change in ER status over time (15). Thus, utilizing the ER status from the primary lesion may not be accurate. Our survey was limited to patients on whom ER status of metastatic disease was known, thus eliminating this possible source of error.

In this study we demonstrated that breast cancer patients with ER-rich metastases respond better to the combination of cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone and survive longer than their ER-poor counterparts. Although prednisone alone may be somewhat more active against hormonally sensitive breast cancer, a comprehensive review by Carter (16) failed to demonstrate any improvement in response or survival when glucocorticoids were added to combination chemotherapy. While some of this improvement in survival is attributable to subsequent response to hormonal therapy in ER-rich patients, a growing body of evidence suggests that breast cancers containing ERs are less aggressive than those lacking them. This has been observed in patients with stage I or II disease (3,4). In addition, Hubay et al (17) have demonstrated that ER-positive patients have a longer disease-free interval following adjuvant chemotherapy than their ER-negative counterparts.

One potential bias in this study is that the cohort was limited to patients with metastatic disease that was amenable to biopsy for ER determination. Thus, both ER-positive and ER-negative groups may be weighted for patients with chest wall or nodal metastasis. Conversely, patients with poor prognosis presentations such as bone marrow, lymphangitic, or central nervous system metastases who do not have accessible tumor for biopsy are excluded. While this unavoidable bias may account, in part, for the unusually long survival after recurrence observed in this series, comparison of these two groups of patients with recurrent disease amenable to biopsy is probably appropriate.

The mechanisms of cytotoxic drug sensitivity and resistance in breast cancer are poorly understood. While this study indicates that those metastatic breast cancers possessing ERs are more responsive to cytotoxic chemotherapy, the molecular determinants for this difference remain to be clarified. Perhaps the promising in vitro cytotoxic drug predictive assays will be of help in further defining subgroups of patients who will respond to cytotoxic drugs and/or hormonal manipulations.

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# Prognostic Factors in Small Cell Carcinoma of the Lung: A Cancer and Leukemia Group B Study [1,2]

L. Herbert Maurer[3,*] and Thomas F. Pajak[4]

## SUMMARY

**Prognostic factors were studied in 224 patients with small cell carcinoma of the lung entered in a Cancer and Leukemia Group B protocol. Extent of disease was the major predictor of complete remission (CR) frequency and survival > 2 years, with CR rates being 40% for patients with limited disease (LD) and 10% for those with extensive disease. Patients who were classified as having extensive disease on the basis of isotope scan evidence of a single bone metastasis had survival characteristics and response rates similar to those of patients with LD. A multivariate analysis of various factors, which may influence overall survival, reveals a good-risk population to be defined as having LD and an initial performance status ≤ 2, with or without a single metastatic focus on bone scan. Furthermore, this risk definition fairly successfully predicted which patients had the greatest chance of achieving a CR (45%). Age > 70 years was shown to be a significantly adverse factor on survival in these multivariate analyses after adjusting for risk. When long-term survivors (> 24 months) were investigated by multivariate analyses, the new risk system was found to be the only significant pretreatment factor. Interestingly, sex was the next marginally influential factor on long-term survival. Future stratification by these risk categories should lead to more homogeneous subgroups for comparison of different therapeutic approaches and should provide some indication as to how much better the newer treatment program is within each risk group.**

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During the past decade, major advances in the management of small cell bronchogenic carcinoma have developed through the use of effective combination chemotherapy and combined modality programs (1–5). These advances have resulted from careful documentation that this disease has certain characteristics that make it amenable to aggressive therapeutic programs. These factors include the findings that a large growth fraction (6,7) makes this disease sensitive to many chemotherapeutic agents and that it tends to disseminate early by hematogenous metastases, thus making it rarely curable by surgery or radiation therapy alone. Survival improvement has been greatest in patients with clinically limited disease (LD), with the potential for achieving a complete remission ranging from 40% to 90% (1,2,4,5). Long-term survival, currently defined as survival > 24 months, is seen in approximately 20% of the complete responders (3). However, patients with extensive disease (ED), while still responsive to chemotherapy, usually do not survive beyond a few months. This failure is due primarily to the inability to induce a sufficient number of complete remissions.

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[3]Department of Medicine, Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Hanover, NH.

[4]Cancer and Leukemia Group B, Scarsdale, NY.

*_Reprint requests to:_ L. Herbert Maurer, MD, Department of Medicine, Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Hanover, NH 03755.

Stage and performance status have been identified as the most important prognostic factors (8). Further analysis of these and other prognostic factors is necessary to better define risk groups so that better therapeutic programs can be designed and uniformly assessed. In this report, we provide data to support a new risk classification of patients which we believe will improve further study of treatment programs in small cell carcinoma of the lung.

## MATERIALS AND METHODS

Cancer and Leukemia Group B (CALGB) protocol 7283 was a randomized trial comparing various combination chemotherapy programs (cyclophosphamide and methotrexate; cyclophosphamide, vincristine, and methotrexate; and cyclophosphamide, vincristine, and high-dose methotrexate with leucovorin rescue) with single-agent chemotherapy (cyclophosphamide) plus radiation therapy for small cell carcinoma of the lung. Patients were entered in this trial between December 1972 and March 1976. The results of this trial have been previously reported (5).

Of the 283 patients entered in this protocol, 25 were excluded from the initial report: ten were ineligible because of wrong tissue type after central pathology review, four had major protocol violations, two died prior to receiving any protocol therapy but after randomization, five had inadequate records, and four were randomized improperly. Also excluded from this current analysis are 33 patients who had incomplete staging data because bone marrow samples were not obtained during the initial staging evaluation, and one patient who had undergone complete surgical removal of the tumor and had no measurable disease. The survival of this latter group of patients is intermediate between that of patients with LD and those with ED (5). Pretherapy staging included physical examination; chest roentgenogram; alkaline phosphatase; SGOT; bone, brain, and liver scans; and bone marrow aspiration or biopsy. Scans were interpreted as normal or suspicious of metastatic disease. Repeat scans were performed at the individual investigator's discretion and were not routinely required. The diagnoses of paraneoplastic syndromes were made on the basis of data accumulated by the investigator at each participating institution.

No patients had received prior chemotherapy or radiation therapy to the primary or metastatic tumor, except for 17 patients who received whole-brain radiation at the start of the protocol because of documented brain metastases.

The following disease sites were examined to determine the relationship of disease extent to survival and response to therapy: primary lung mass, mediastinal and hilar lymph nodes, supraclavicular lymph nodes, other lymph nodes, brain, liver, bone, skin, and bone marrow. No patients with superior vena cava syndrome were entered in this protocol. The patients were evaluated for objective tumor response to the combined modality program, and each patient was classified as having a complete response (CR), partial response, improvement, no change, or disease progression, as defined previously (5). For the present report, only complete responders (ie, patients who had complete regression of all measurable lesions and disappearance of all other objective parameters or active disease including signs of ectopic hormone production) are analyzed, as these are the patients who enter long-term remission. No systematic reassessments for CR at specific time points were part of the protocol. Performance status criteria were defined according to the following CALGB classification: 0 (normal activity); 1 (ambulatory); 2 (< 50% of the time in bed); 3 (> 50% of the time in bed); and 4 (bedridden).

### Statistical Methods

Various differences were evaluated using the Pearson chi-square test (9) and the Kruskal-Wallis test (10). Survival differences were evaluated by the Breslow modification of the Kruskal-Wallis test (11). Screening of pretreatment characteristics and treatment components that might influence CR frequency and survival was performed with the linear logistic model (12) and the Cox regression model (13).

## RESULTS

### Extent of Disease

Differences were noted in survival and ability to achieve a CR between patients with LD and those with ED. In patients with disease limited to the lung and mediastinum, the median survival was 9.2 months, and 40% achieved a CR. Metastatic spread to the ipsilateral, contralateral, or bilateral supraclavicular lymph nodes did not alter the prognosis (table 1). The median survival for the complete responders was 12.4 months, with 20% entering a long-term remission. The median survival for patients with ED (spread beyond the above sites) was 4.6 months, significantly different from that for patients with LD ($P = 0.001$). Similarly, the ability to achieve a CR was reduced to only 10%. In patients with ED, the site of metastatic spread influenced the percent of patients achieving a CR (table 1). Patients with hepatic or cutaneous metastases

TABLE 1.—Survival by extent of disease

| Disease extent | No. of patients | Median survival in mos, all | % with CR | Median survival in mos, complete responders | % of complete responders alive > 24 mos |
|---|---|---|---|---|---|
| Disease confined to 1 lung and mediastinum | | | | | |
| Supraclavicular node negative | 92 | 9.1 | 40 | 12.4 | 14.0 |
| Supraclavicular node positive | 22 | 9.7 | 41 | 9.7 | 35.2 |
| ED | 110 | 4.7 | 10 | 12.5 | 1.1 |
| Liver | 63 | 4.6 | 3 | 9.5 | 0 |
| Skin | 5 | 3.5 | 0 | — | — |
| Brain | 17 | 3.5 | 6 | 16.0 | 0 |
| Bone marrow | 39 | 3.7 | 10 | 12.5 | 0 |
| Bone (all) | 46 | 5.3 | 17 | 11.5 | 1.6 |
| Bone (single lesion) | 7 | 11.5 | 71 | 12.5 | 14.0 |
| Bone (adjusted for single lesion) | 39 | 4.7 | 8 | 10.0 | 0 |
| ED (adjusted for single bone lesion) | 103 | 4.6 | 6 | 12.0 | 0 |

seldom achieved a CR, while patients with brain and bone marrow metastases appeared to do slightly better in terms of remission induction. Patients with bone metastases appeared to do best, achieving a 17% CR rate. However, further analysis of these patients indicated that seven of 46 patients were classified as having ED solely on the basis of a single defect seen on bone scan or roentgenogram: metastases were adjacent to the primary tumor in four patients and at distant sites in three. This small group of patients appeared to do exceptionally well, with 71% achieving a CR and having survival characteristics similar to patients with LD. Except for this group with single bone metastases, the slight variations in CR between metastatic sites were not translated into substantial differences in median survival.

## Performance Status

Performance status was the second major predictor for achieving a CR (tables 2 and 3). In patients with LD, only those with severe performance changes (3 or 4) had a reduction in ability to achieve a CR. However, long-term survival for complete responders in patients with LD was decreased with increasing impairment of performance status. In patients with ED, only those with a performance score of 0 have a significant chance of achieving a CR (table 2). When patients with a single bone lesion are excluded from the analysis of ED (table 3), the CR rate even for patients with performance scores of 0 is quite low at 14%.

## Age

Patients > 70 years of age do not appear to achieve CR to the combined modality program as often as do those < 70 years old, particularly if they had LD ($P = 0.38$). In patients with ED, little difference in CR rates was seen; however, the median survival for patients > 70 years old is less than half the time of those younger ($P = 0.10$). Median survival of complete responders was not affected by age; however, no long-term survivors were seen in the older age group. Although long-term survivors were predominantly < 60 years of age (table 2), this difference was not statistically significant.

## Sex

No difference in median survival or ability to achieve a CR was seen between males and females (table 2). However, once a CR was achieved, women with LD appeared more likely to survive 2 years than men (35.1% vs 15.4%, $P = 0.08$). The one long-term survivor with ED was a woman with a single bone lesion as the sole basis for this stage classification.

## Paraneoplastic Syndromes

Seventeen patients were reported to have one or more paraneoplastic syndromes (table 4). The presence of these metabolic or neurologic abnormalities appeared to have an adverse effect on the CR rate and survival (table 2).

TABLE 2.—Prognostic features with small cell carcinoma of the lung

| | Patients with LD | | | | | Patients with ED | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total evaluable | Median survival in mos, all | % with CR | Median survival in mos, complete responders | % of complete responders alive > 24 mos | Total evaluable | Median survival in mos, all | % with CR | Median survival in mos, complete responders | % of complete responders alive > 24 mos |
| Performance score | | | | | | | | | | |
| 0 | 48 | 10.7 | 42 | 13.0 | 27.5 | 26 | 8.3 | 27 | 15.0 | 1.8* |
| 1–2 | 51 | 8.2 | 43 | 9.6 | 11.4 | 56 | 4.3 | 4 | 9.5 | 0 |
| 3–4 | 9 | 5.3 | 11 | 11.0 | 0.0 | 21 | 3.8 | 5 | 12.0 | 0 |
| Age (yrs) | | | | | | | | | | |
| < 60 | 51 | 10.3 | 45 | 12.0 | 23.9 | 55 | 4.9 | 11 | 12.0 | 2.1* |
| 60–69 | 48 | 8.5 | 40 | 10.2 | 15.0 | 42 | 5.0 | 10 | 14.5 | 0 |
| 70+ | 15 | 9.3 | 27 | 13.0 | 0 | 13 | 2.3 | 8 | 12.0 | 0 |
| Sex | | | | | | | | | | |
| Male | 90 | 9.2 | 40 | 10.8 | 15.4 | 93 | 4.8 | 11 | 12 | 0 |
| Female | 24 | 10.0 | 42 | 19.0 | 35.0 | 17 | 4.3 | 6 | 25 | 100* |
| Paraneoplastic syndromes present | 6 | 6.0 | 0 | 0 | 0 | 11 | 3.5 | 0 | 0 | 0 |
| Location | | | | | | | | | | |
| Right | 58 | 8.9 | 38 | 12.0 | 22.1 | 41 | 4.8 | 10 | 12.0 | 3.1* |
| Left | 46 | 9.7 | 43 | 10.8 | 17.7 | 57 | 4.3 | 9 | 14.0 | 0 |

*1 female patient with a single bone metastasis.

## Miscellaneous

Pulmonary location of the primary tumor (left vs right) had no influence on survival or ability to achieve a CR (table 2).

## Multivariate Analysis of Risk Factors on Survival

The first 11 factors in table 5 were used in the Cox regression model to identify pretreatment factors that may have a significant influence on survival. Two definitions for extent of disease were next analyzed in addition to the other 11 factors to determine whether one of the definitions is a significant factor in its own right. CR outcome was then added to the other 13 factors, not only to confirm the importance of achieving a CR, but also to determine whether it reduces the influence of all other factors on survival to the point of statistical nonsignificance. Finally, good- versus poor-risk status was considered to be a separate predictor of

TABLE 3.—Performance status by adjusted stage*

| | Performance score | No. of patients evaluable | Median survival in mos, all | % with CR | Median survival in mos, complete responders | % of complete responders alive > 24 mos |
|---|---|---|---|---|---|---|
| Adjusted LD | 0 | 52 | 10.9 | 46 | 14.0 | 25 |
| | 1–2 | 54 | 8.0 | 43 | 9.8 | 11 |
| | 3–4 | 9 | 5.3 | 11 | 11.0 | 0 |
| Adjusted ED | 0 | 22 | 7.0 | 14 | 14.5 | 0 |
| | 1–2 | 53 | 4.3 | 2 | 7 | 0 |
| | 3–4 | 21 | 3.8 | 5 | 11 | 0 |

*Patients with single scan evidence of a bone metastasis as the sole basis for classifying them as having ED were considered to have LD.

TABLE 4.—Paraneoplastic syndromes

| Syndrome | No. of patients |
|---|---|
| Eaton-Lambert syndrome | 4* |
| Syndrome of inappropriate antidiuretic hormone (SIADH) | 6* |
| Hypercalcemia | 2† |
| Cushing's syndrome with elevated plasma adrenocorticotropic hormone | 3 |
| Dermatomyositis | 1 |
| Peripheral neuropathy and cerebellar degeneration | 1 |
| Nephrotic syndrome and subacute cerebellar degeneration | 1 |

*1 patient had both SIADH and Eaton-Lambert syndrome.
† Bone and bone marrow metastases.

survival. Good risk was defined as disease limited to the lung, mediastinum, and supraclavicular lymph nodes, with or without a single metastatic focus on bone scan, and an initial performance score ≤ 2. All other patients were considered to be poor-risk. Results of the analysis outlined above are summarized in table 6.

The differences in the log likelihood values associated with the fitted Cox's regression models at steps 1 and 2 is 6.00, which is statistically significant at $P = 0.014$. Hence, the addition of modified disease extent led to an improved fit of the regression model. The modified disease extent definition represents a statistically better discriminant of survival than the usual definitions of disease extent ($P = 0.032$).

TABLE 5.—Factors analyzed for potential effect on survival

| Factor No. | Factors | Choices |
|---|---|---|
| 1 | Liver involved | Yes vs no |
| 2 | Marrow involved | Yes vs no |
| 3 | Bone involved | Yes vs no |
| 4 | Brain involved | Yes vs no |
| 5 | Node involved | Yes vs no |
| 6 | Hormone | Abnormal vs normal |
| 7 | Sex | Male vs female |
| 8 | Age | Continuous |
| 9 | Age groups | < 60 yrs vs 60–69 yrs vs 70+ yrs |
| 10 | Initial performance scores grouped | 0 vs 1+2 vs 3+4 |
| 11 | Location of primary | Left vs right |
| 12 | Extent of disease | LD vs ED |
| 13 | Modified extent of disease* | Adjusted LD vs adjusted ED |
| 14 | CR outcome | No vs yes |
| 15 | Risk group | Good vs poor |

*Patients with only a single bone metastasis as the sole basis for classifying them as having ED were considered to have LD.

When the CR outcome was added to the model at the third step, it proved to be the most significant variable. However, three other factors still remain as significant factors influencing survival. They were modified disease extent, grouped initial performance scores, and grouped ages. Finally, when the new risk classification scheme was added at step 4, it proved to be the most significant factor, even greater than CR outcome. The difference between the fitted models at steps 3 and 4 is not statistically significant ($P = 0.09$). However, risk is associated with a significantly better linear logistic model in predicting CR ($P < 0.001$) (table 7). This is not surprising when one compares the magnitude of the differences in CR frequencies between the LD and the ED groups (40% – 10% = 30%) and between the good- versus the poor-risk groups (45% – 6% = 39%) (table 7).

Long-term survival (> 24 months from start of therapy) was investigated through the use of the linear logistic model. All of the pretreatment factors listed in table 5, excluding CR outcome, were initially considered. The only factor found to significantly influence long-term survival was whether the patient was a good or poor risk ($P = 0.001$). When CR outcome was added to the other factors in table 5, it turned out to be the only statistically significant factor ($P < 0.001$). Interestingly, sex was the next marginally influential factor on long-term survivals ($P = 0.07$). Figure 1 indicates that relapses continue beyond 24 months, with only 4% of the good-risk patients alive at 4 years.

## DISCUSSION

The extent of disease in small cell carcinoma of the lung has been well-recognized as the major prognostic feature. The Veterans Administration Lung Cancer Study Group first proposed the staging system generally in use today (14,15). This system defines LD as tumor confined to one hemithorax, the mediastinum, and ipsilateral biopsy-positive scalene nodes, but without palpable supraclavicular nodes or masses. Disease spread beyond these sites was classified as ED. These important early studies helped to define the natural course of the disease in patients being randomized to radiation therapy, chemotherapy, or placebo. In the untreated patients, the median survival was slightly greater than 3 months for patients with LD and was 1.4 months for those with ED. Most current studies employ this staging classification (1–5), although the majority include palpable ipsilateral supraclavicular lymph nodes in the LD stage. The present study again confirms that division of the patient population into

TABLE 6.—Results of multivariate analyses of factors influencing survival

| Stage No. | Factors considered (table 5) | Significant factors | *P* value |
|---|---|---|---|
| 1 | 1–11 | Liver involvement (no > yes) | < 0.001 |
| | | Initial grouped performance scores (0 > 1+2 > 3+4) | < 0.001 |
| 2 | 1–13 | Modified disease extent (adjusted LD > adjusted ED) | < 0.001 |
| | | Initial grouped performance scores (0 > 1+2 > 3+4) | < 0.001 |
| 3 | 1–14 | CR (yes > no) | < 0.001 |
| | | Modified disease extent (adjusted LD > adjusted ED) | < 0.001 |
| | | Initial grouped performance scores (0 > 1+2 > 3+4) | < 0.001 |
| | | Grouped ages (< 60 yrs > 60–69 yrs > 70+ yrs) | 0.031 |
| 4 | 1–15 | Risk (good > poor) | < 0.001 |
| | | CR (yes > no) | < 0.001 |
| | | Initial grouped performance scores (0 > 1+2 > 3+4) | 0.002 |
| | | Grouped ages (< 60 yrs > 60–69 yrs > 70+ yrs) | 0.026 |

TABLE 7.—Response and survival in small cell carcinoma of the lung as a result of risk definition

| Category | No. of patients | Median survival in mos, all | % with CR | Median survival in mos, complete responders | % of complete responders alive > 24 mos |
|---|---|---|---|---|---|
| Good risk | 112 | 10.0 | 45 | 12.5 | 19.5 |
| Poor risk | 112 | 6 | 6 | 11.5 | 0 |

LD versus ED groups has major prognostic significance, not only in terms of survival but in the ability to achieve significant therapeutic response. Further, our data suggest that bilateral or contralateral supraclavicular lymphatic spread does not alter the prognosis, and patients with disease spread to these sites should be included in the LD stage.

In patients with ED, the initial site of metastases had an influence on response rate and survival. Patients who presented with liver, brain, or skin metastases had an extremely poor prognosis, with only 3.6% achieving a CR. The observation that patients with liver and brain metastases have an extremely poor prognosis and shortened survival has been documented by Lanzotti et al (16). Patients with bone or bone marrow metastases had a slightly better prognosis, with 14% of these patients achieving a CR. In patients with a single suspicious area of increased isotope uptake on bone scan, there was no unfavorable impact on ability to achieve a CR, with 71% achieving a CR. It is not clear whether these areas represented metastatic disease. Therefore, care must be given in the future to document spread by biopsy if a single bone scan defect is observed and is the only evidence for ED.

Performance score at diagnosis has a direct effect on remission rate. In patients with LD, no deleterious influence on ability to achieve a CR was seen until marked deterioration in the initial performance score was observed. Despite the good remission rates in patients with LD and performance scores $\leq 2$, the 2-year survival rates were affected by increasing disability. In patients with ED, if single bone metastases are not included in the analysis,

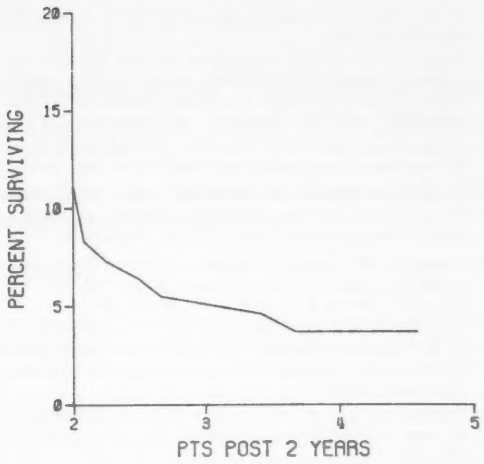


FIGURE 1.—Survival of all good-risk patients after 2 yrs.

any initial performance score > 0 had a profound negative influence on remission rate, with only two of 75 patients achieving a CR. Performance status evaluation as a predictor of response in small cell carcinoma of the lung has been reviewed by Hansen et al (8) and Lanzotti et al (16).

Age and sex, when considered individually, were not identified as possible prognostic variables. Age, when considered in various multivariate analyses, was shown to significantly influence survival. For example, in the poor-risk patients, the median survival for patients > 70 years old was 2.0 months, as compared to 4.7 months for patients < 50 years old and 5.3 months for patients between 60 and 69 years old ($P = 0.03$). While sex does not play a role in the ability to attain a CR, there is a trend toward greater long-term survival in females. Lagakos (17) reported a superior survival for females in the Eastern Cooperative Oncology Group's study, but long-term survival was not reported. These differences in long-term survival are of interest in view of the recent reports of estradiol production by tumor cell lines derived from small cell carcinoma of the lung (18) and a decreased incidence of tumor takes when small cell carcinoma was inoculated into female nude mice as compared with male nude mice (19). Whether this survival benefit is related to complex hormonal and immunologic interactions will need to be studied further.

No patients who had clinically detectable metabolic or neurologic abnormalities achieved a CR. This influence of paraneoplastic syndromes on CR and survival has not previously been reported. The frequency of clinical detection of paraneoplastic syndromes has varied from 2.8% to 26% (20). Radioimmunoassays have detected increased levels of adrenocorticotropic hormone, neurophysins, calcitonin, and other hormones in most patients; these hormones may prove of value in the future as markers of therapeutic response (20). One study suggests that there may be differences in secretory status between patients with LD and those with ED (21).

The multivariate analysis attempts to combine the major prognostic features into a system of good or poor risk, which further refines our ability to predict which patients have the greatest chance for achieving a CR and thus have an increased probability for long-term survival. Future stratification into these categories should lead to more homogeneous subgroups for comparison of different therapeutic approaches. One can then be better able to distinguish whether the higher reported response rates or higher percent of long-term survivors are the result of improved treatment programs or of selection of patients. It can also provide an idea of how much better the newer treatment program is within each group. Further refinement of this new risk system may occur after analysis of more active therapy programs.

Future investigational therapeutic questions in small cell carcinoma must be addressed by disease extent. In the poor-risk patients, the primary goal should be to find new treatment strategies to increase the CR frequency from the 6% seen in this study. In the good-risk patients, there should be two major goals. The first is, again, to find new strategies to increase the CR rate, and the second is to reduce the high local-regional recurrence rate in the complete responders. To design new treatment strategies that simultaneously attack these two questions in this patient group is unrealistic.

The current approach by the CALGB has been to design studies in the good-risk patients to specifically test different types of local control and their timing. In the poor-risk patients, the emphasis will be placed on testing new treatment strategies in an attempt to increase the response frequency. This approach optimizes the available resources in finding treatments that would significantly increase the percent of 2-year survivors in the good-risk group and, hopefully, achieve a significant number of disease cures.

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# Vindesine in the Treatment of Refractory Breast Cancer: Improvement in Therapeutic Index With Continuous 5-Day Infusion[1,2,3]

**Hwee-Yong Yap,* George R. Blumenschein, Gerald P. Bodey, Gabriel N. Hortobagyi, Aman U. Buzdar, and Alfred DiStefano[4]**

## SUMMARY

**Sixty patients with metastatic breast cancer refractory to prior doxorubicin combinations were randomized by performance status, dominant disease site, and number of involved organ sites to receive vindesine either as a bolus injection of 3–4 mg/m² iv every 10–14 days or as a continuous 5-day infusion of 1–1.2 mg/m²/day every 21 days. There were two patients with partial responses (7%) and six with stable disease among the 26 evaluable patients who received bolus injections. Of the 25 evaluable patients who received continuous infusions, seven achieved a partial response (28%) and 11 had stable disease ($0.001 < P < 0.005$). Thirteen patients, after failing to respond to bolus vindesine, were given continuous infusions. Of these, 11 were evaluable and four had partial responses (36%). Responses were seen in all organ sites of involvement, with response duration ranging from 2 to 9+ months. Side effects included nausea, vomiting, stomatitis, constipation, neuropathy, fever, and myelosuppression. Except for myelosuppression, which was more evident with the continuous infusion schedule, no significant difference was seen in the frequency of side effects encountered with the two schedules. These results confirmed that there is an improved therapeutic index for vindesine when it is given as a continuous 5-day infusion.**

[Cancer Treat Rep 65:775–779, 1981]

Vindesine is a semisynthetic vinca alkaloid derived from vinblastine. It was chosen for clinical evaluation because its spectrum of antitumor activity is wider than that of vinblastine in animal tumor test systems and its toxicity is intermediate between that of vincristine and vinblastine (1,2). Early phase I–II clinical studies revealed evidence of antitumor activity in a variety of tumors when vindesine was given as bolus injections of 2–4 mg/m² every 1–2 weeks (3–8). Preliminary clinical data suggested an improved therapeutic index when it is given as a continuous iv infusion in place of the bolus iv doses (8). This led us to conduct a phase II evaluation to compare the therapeutic efficacy and toxicity of a continuous 5-day infusion with those of bolus injection of vindesine in patients with metastatic breast cancer.

## PATIENTS AND METHODS

Sixty patients with histologically proven breast cancer who had failed prior therapy with combinations containing doxorubicin were entered in this study. All patients were randomized by performance status, number of involved organ sites, and

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[4]The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to*: Dr. Hwee-Yong Yap, Department of Medicine, Medical Breast Service, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

dominant disease site to receive vindesine either as a bolus injection or as a continuous 5-day infusion. Based on our phase I studies (3), vindesine was administered at a dose of 4 mg/m$^2$ as a single iv injection repeated at approximately 10-day intervals in patients who received the bolus treatment. In patients with significant liver dysfunction (bilirubin > 2 mg/100 ml) or in those with poor bone marrow reserve, a reduced dose of 3 mg/m$^2$ was given. The first five patients entered in the continuous 5-day infusion schedule were given vindesine at doses of 1.4 mg/m$^2$/day. Subsequently, because of excessive myelotoxicity, all patients were entered at a dose of 1.2 mg/m$^2$/day for 5 days. The dose was reduced to 1.0 mg/m$^2$ for 5 days in those patients with significant liver dysfunction (bilirubin > 2 mg/100 ml) and in those with poor bone marrow reserve. To avoid the risk of tissue necrosis due to extravasation, central venous delivery with an indwelling silicon elastomer venous catheter was used in all patients who received continuous infusions. Whenever it was feasible, the catheter was inserted via a peripheral vein rather than via the subcutaneous subclavian approach. To maintain a steady rate of infusion, McGaw volumetric infusion pumps were used for all inpatients and Autosyringe infusion pumps were used for outpatients. The doses used for both schedules were modified during the study to maintain a tolerable degree of hematologic (absolute granulocyte count, 750–1500/mm$^3$; platelet count, 75,000–100,000/mm$^3$) and nonhematologic toxic effects.

Patients had complete evaluations, including history and physical examination, total hematology survey, blood chemistry determinations, urinalysis, and appropriate radiologic and isotopic examinations. Tumor measurements were documented prior to each course of chemotherapy. Informed written consent was obtained from all patients prior to the start of treatment. Patients were evaluable for response only if they had received more than one course of vindesine chemotherapy, except when there was evidence of development of new lesions following the first course of therapy with continuous infusions. These patients were considered to have disease progression. Partial response was defined as a 50% reduction of the sum of the products of all measurable lesions without the appearance of new lesions, plus recalcification of osseous lytic lesions. In the case of malignant hepatomegaly, partial response was defined as either a 3-cm reduction in hepatomegaly or a 50% reduction in the summation of liver enlargement below the costal margin in both midclavicular lines plus the epigastric line. Stable disease was defined as a < 25% increase or decrease in measurable disease, with less than partial response for at least 8 weeks and without the appearance of new lesions. Disease progression was defined as a > 25% increase of measurable disease or the appearance of new lesions. Time to disease progression was calculated from the date chemotherapy was started to the first documentation of disease progression, using the Kaplan and Meier method (9). Statistical analysis of the differences between the curves was made by the two-tailed generalized Wilcoxon test modified by Gehan (10).

## RESULTS

Of the 30 patients entered in the continuous 5-day infusion schedule, three died early (within 2 weeks from the start of treatment), one was a major protocol violator (received the first course as continuous infusion and subsequent courses as bolus injections daily × 5 every 3 weeks), and one had an inadequate trial, having received only one course of therapy, and was subsequently lost to followup. Of the 30 patients entered in the bolus schedule, one died early and three had inadequate trials, having received only one course of chemotherapy, with no evidence of disease progression. Table 1 shows some

TABLE 1.—Patient characteristics*

| Characteristic | Continuous infusion | Bolus injection |
|---|---|---|
| No. of patients | 25 | 26 |
| Age (yrs) | | |
| Median | 54 | 55 |
| Range | 30–75 | 33–75 |
| Disease-free interval (mos) | | |
| Median | 24 | 21 |
| Range | 1–168 | 0–156 |
| Menopausal status | | |
| Premenopausal | 2 | 3 |
| Postmenopausal | 20 | 21 |
| Unknown | 3 | 2 |
| Median duration of prior therapy (mos) | 23 | 22 |
| Prior treatment | | |
| Median | 3 | 3 |
| Range | 1–7 | 1–7 |
| Dominant disease | | |
| Soft tissue | 3 | 2 |
| Osseous | 6 | 6 |
| Visceral | 16 | 18 |
| Sites of disease | | |
| 1–2 | 17 | 18 |
| ≥ 3 | 8 | 8 |
| Performance status† | | |
| 0–1 | 14 | 15 |
| ≥ 2 | 11 | 11 |

*Unless otherwise indicated, values = No. of patients.
†International Union Against Cancer.

of the pertinent patient characteristics. No significant difference was seen in the distribution of patients on the two treatment schedules with regard to age, disease-free interval, number of organ sites involved, dominant disease site, performance status, number of prior treatments, and duration of prior therapy. Of the 25 patients who received continuous infusions, there were seven partial responders, giving a response rate of 28%. Eleven patients had stable disease and seven had disease progression in spite of treatment. Of the 26 patients who received the bolus treatment, two achieved a partial response, giving a response rate of 7%. Six patients had stable disease and 18 had disease progression. No statistical difference between the two different treatments was seen when the responders and the nonresponders were compared ($0.1 < P < 0.25$). When no responses were observed in the first 13 patients randomized to receive bolus injections of vindesine, the subsequent additional 13 patients who had been entered in the bolus schedule were switched from bolus to continuous infusions (11 upon evidence of disease progression and two who had no response). One patient died early, and one had an inadequate trial (she developed thrombosis of the subclavian vein after one course of continuous infusion with vindesine), but the remaining 11 patients were evaluable for response. Four of these patients subsequently achieved partial response with the continuous infusions, having previously demonstrated disease progression while receiving bolus injections. Thus, including the 11 patients who first received bolus injections and then received continuous infusions, a total of 36 evaluable patients received continuous infusion with vindesine. Among these 36 patients, there were 11 partial responders, with an overall response rate of 30%. Table 2 shows the response by site of metastasis. Except for soft tissue and osseous disease, no response was seen in any of the major sites of organ involvement in patients treated with bolus injections. Soft tissue and lymph nodes appeared to be the most responsive metastatic sites when continuous 5-day infusion was used, but responses were seen in all major sites of organ involvement except for malignant pleural effusion (measurable if complete resolution occurred with treatment). Responses were evident at a median of one course of therapy (range, one to three).

TABLE 2.—Response by metastatic site*

| Organ site | Continuous infusion | Bolus injection |
|---|---|---|
| Soft tissue | 3/9 (33) | 2/14 (14) |
| Lymph node | 3/5 (60) | 0/5 (0) |
| Lung | 1/5 (20) | 0/4 (0) |
| Pleura | 0/8 (0) | 0/11 (0) |
| Bone | 4/17 (23) | 1/15 (6) |
| Liver | 1/7 (14) | 0/8 (0) |

*Values = No. of patients responding/total No. of patients (%).

TABLE 3.—Hematologic toxicity

| | Continuous infusion | Bolus injection |
|---|---|---|
| Wbc count nadir (cells/mm$^3$ × 10$^3$) | | |
| Median | 2.0 | 2.9 |
| Range | 0.9–4.7 | 1.0–8.9 |
| Absolute granulocyte count nadir (cells/mm$^3$ × 10$^3$) | | |
| Median | 0.9 | 1.5 |
| Range | 0.1–3.0 | 0–6.3 |
| Absolute platelet count nadir (cells/mm$^3$ × 10$^3$) | | |
| Median | 185 | 156 |
| Range | 51–475 | 14–378 |
| % of courses with absolute granulocyte counts ≤ 1000/mm$^3$ | 61 | 30 |
| % of courses with absolute granulocyte counts ≤ 500/mm$^3$ | 29 | 10 |

The toxic effects associated with the administration of both vindesine schedules are summarized in tables 3 and 4. Myelosuppression observed with the bolus schedule was moderate, transient, and noncumulative. In contrast, severe myelosuppression was observed at a dose of 1.4 mg/m$^2$/day for 5 days with continuous infusions. Only the first five pa-

TABLE 4.—Nonhematologic toxicity*

| | Continuous infusion | Bolus injection |
|---|---|---|
| No. of evaluable patients | 25 | 26 |
| No. of patients with (%)— | | |
| Nausea | 8 (32) | 21 (80) |
| Vomiting | 6 (24) | 18 (69) |
| Stomatitis | 5 (20) | 5 (20) |
| Fever with infusion | 8 (32) | 2 (7) |
| FUO with neutropenia | 4 (16) | 2 (7) |
| Phlebitis | — | 2 (7) |
| Alopecia | 1 (4) | 1 (3) |
| Neuropathy | | |
| Constipation | 6 (24) | 15 (57) |
| Ileus | 1 (4) | 1 (3) |
| Paresthesia | 5 (20) | 7 (27) |
| Loss of deep tendon reflexes | 2 (8) | 2 (7) |
| Muscular weakness | 2 (8) | 0 |
| Inappropriate ADH secretion | 4 (16) | 0 |
| Catheter-related | | |
| Pneumothorax | 1 (4) | 0 |
| Thrombosis | 2 (8) | 0 |
| Extravasation | 1 (4) | 2 (7) |

*FUO = fever of unknown origin; ADH = antidiuretic hormone.

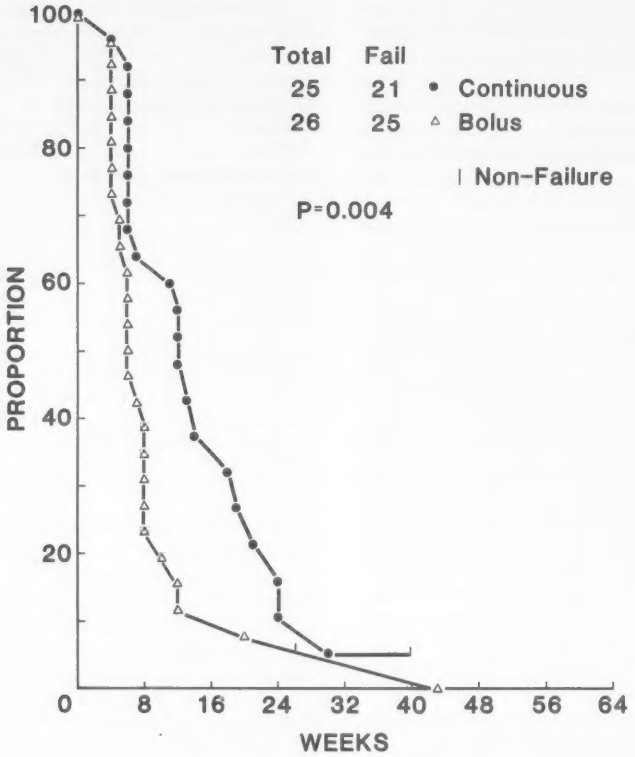


FIGURE 1.—Time to disease progression in patients receiving vindesine.

tients entered in the continuous infusion schedule received a first course of therapy at this dose level. All patients were subsequently entered at doses of 1.0–1.2 mg/m²/day for 5 days. At these reduced doses, the degree of myelosuppression observed was slightly more severe than with the bolus treatment.

Although nausea, vomiting, and constipation were less frequently encountered with continuous infusions, no significant difference was seen in the frequency of stomatitis, paralytic ileus, and peripheral neuropathy with the two schedules. Fever during infusion was experienced by 32% of the patients receiving vindesine as continuous infusions, compared with 7% of those who received bolus injections. The syndrome of inappropriate antidiuretic hormone secretion was diagnosed in four patients receiving vindesine; there were other possible contributing factors, including iv hyperalimentation, sepsis, and antibiotic therapy. Thus, the occurrence of this syndrome may not have been solely related to the administration of vindesine.

Figure 1 shows the time to disease progression for all patients. The median time to disease progression was 6 weeks (range, 4–43) for all patients who received bolus injections, and that is significantly shorter ($P = 0.004$) than the median time to disease progression of 12 weeks (range, 4–40+) for all patients receiving continuous infusions.

## DISCUSSION

The 7% response rate observed with the bolus schedule of vindesine in the treatment of patients with metastatic breast cancer who had previously received extensive chemotherapy is comparable with that previously described by other investigators (7) but appeared to be inferior to the 27% overall response rate observed with continuous 5-day infusions. The administration of vindesine by both schedules was moderately well-tolerated by most patients, although there was a lower incidence of nausea, vomiting, and constipation with continuous

infusions. In accordance with earlier phase I–II studies (3–8), the occurrence of peripheral neuropathy was the most significant dose-limiting factor with both schedules. The degree of myelosuppression observed was slightly more severe in all patients who received continuous infusions. The myelosuppression observed with the bolus schedule was moderate, transient, and noncumulative. This led us to attempt further increase in dose, which was unsuccessful because of prolonged myelosuppression resulting in a lengthening of intervals between courses of therapy. The higher response rate observed with continuous 5-day infusions, without added toxicity, suggests an improved therapeutic index of vindesine when it is given as a continuous 5-day infusion. Over a 3-week period of therapy, patients given the drug by the bolus schedule received a total dose of at least 8 mg/m$^2$ of vindesine, compared with a total dose of 6 mg/m$^2$ for those patients who received continuous infusions. It is, therefore, unlikely that the better therapeutic results observed with the continuous infusion schedule are dose-related.

Mathé et al (8) recently reported an improved therapeutic index when vindesine was delivered as a continuous 24–48-hour infusion in patients with acute leukemia and blast crisis chronic myelogenous leukemia. Similar to their observation, four of our 11 patients who had demonstrated prior refractoriness to the bolus schedule of vindesine subsequently had partial responses with the continuous infusion schedule. These results further support the contention that the therapeutic index of vindesine is augmented when it is delivered by the continuous infusion schedule and that the index is similar to that seen in our earlier observation with vinblastine (11).

Although continuous infusion with vincristine has been previously studied (12,13), the design of these studies precludes any meaningful comparative evaluation of therapeutic efficacy of conventional bolus injection of vincristine. Nevertheless, available preclinical (14,15) and clinical data suggest that the vinca alkaloids may be schedule-dependent and that further studies may be worthwhile to clearly delineate the schedule dependency and the comparative therapeutic efficacy of the three vinca alkaloids.

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# Cisplatin, Vindesine, and Bleomycin (DVB) Combination Chemotherapy for Esophageal Carcinoma[1,2]

D. P. Kelsen,* Manjit Bains, Robert Chapman, and Robert Golbey[3,4]

## SUMMARY

**Forty-six patients with epidermoid carcinoma of the esophagus have been treated with a three-drug combination of cisplatin, vindesine, and bleomycin. Of the 40 patients currently evaluable for response, 21 have had partial remissions (52%). At least four of these responses were almost complete, with only microscopic disease found on endoscopy or review of the resected specimen. Toxic effects have, in general, been manageable. The major toxic effects included nausea and vomiting, nephrotoxicity, and myelosuppression. There were two drug-related deaths: one due to renal failure and one due to sepsis. The three-drug combination appears to be substantially more effective than either the two-drug combination of cisplatin and bleomycin or vindesine alone. Effects on survival cannot yet be evaluated.**

[Cancer Treat Rep 65:781–785, 1981]

The treatment of patients with esophageal carcinoma remains one of the more difficult problems in cancer therapy. Even when the disease is diagnosed relatively early, aggressive surgical and radiation therapy techniques still yield 5-year survival rates of < 20% (1). Most patients present with disease too advanced for potentially curative therapy. Chemotherapy has, in general, been ineffective. Bleomycin, the most well-studied single agent, induces complete and partial responses in 10%–15% of patients, with a median response duration of 1–2 months (2–5); it is generally believed to be of little clinical benefit, even to most responding patients. Two studies from Memorial Hospital have recently been reported. In the first, the combination of cisplatin (DDP) and bleomycin was shown to induce complete and partial responses in 19% of the patients, with minor regression seen in an additional 44% (6). In patients with metastatic disease, the response rate was 23% and the median duration of remission was 6 months. In the second trial, vindesine (VDS) was studied as a single agent; a complete and partial response rate of 18% was noted. Among the responders were several patients who had relapsed following multiple cycles of DDP and bleomycin (7). The median duration of response was 5 months. In both studies, the median survival of nonresponding patients was 2 months. In view of the apparent lack of cross-resistance, differing mechanisms of action, and different toxic effects, the current study was designed to evaluate the efficacy of DDP, VDS, and bleomycin as a three-drug combination (DVB).

## MATERIALS AND METHODS

Forty-six patients with histologic or cytologic proof of epidermoid carcinoma of the esophagus have been entered in this study, which was activated in July 1979. Characteristics of the patient population are shown in table 1; although the median performance status (PS) on the Karnofsky scale was 70, four

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[3]Departments of Medicine (Solid Tumor Service) and Surgery (Thoracic Surgery Service), Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY.

[4]We thank Barbara Nagerl for preparation of the manuscript and Mary Dukeman for assistance with analysis of the data.

**Reprint requests to:* David Kelsen, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

TABLE 1.—Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Total | |
| Entered | 46 |
| Evaluable | 40 |
| Inevaluable | 3 |
| Pending | 3 |
| Age (yrs) | |
| Median: 62 | |
| Range: 42–73 | |
| PS | |
| Median: 70 | |
| Range: 30–90 | |
| Sex | |
| Males | 33 |
| Females | 13 |
| Prior therapy | |
| None | 39 |
| Chemotherapy | 2 |
| Irradiation | 5 |

TABLE 2.—Sites of indicator lesions

| Site | No. of patients |
|---|---|
| Esophagus | 41 |
| Lymph node | 9 |
| Lung | 3 |
| Liver | 3 |
| Soft tissue | 2 |

patients had a pretreatment PS < 40. Thirty patients had disease clinically limited to the local-regional area (no evidence of invasion of the bronchotracheal tree and no evidence of distant metastases) and were candidates for a combined-modality approach. Neither the length of the primary lesion nor the degree of obstruction was considered to preclude entrance in this arm of therapy. These patients received a single course of chemotherapy prior to surgery or two courses prior to radiation therapy; response to chemotherapy was measured by repeat barium esophagram. Sixteen patients had proven metastatic or recurrent disease and were treated with chemotherapy alone. All patients had measurable lesions, either by barium esophagram or by bidimensionally measurable metastases. Sites of indicator lesions are shown in table 2.

Pretreatment evaluation included a complete history and physical examination, barium esophagram, chest roentgenogram, 12-channel screening profile, cbc and platelet count, ECG, creatinine clearance, and pulmonary function tests (PFT). All patients with esophageal lesions located at the level of the carina or higher underwent bronchoscopy; gross invasion of the trachea or bronchus was considered a contraindication to therapy with DVB. Appropriate nuclide scans, computerized tomograms, and audiograms were obtained as clinically indicated. Interval studies included a cbc and platelet count before each chemotherapeutic treatment, a creatinine clearance prior to each course of DDP, and PFT before the second course of bleomycin. A barium esophagram to evaluate response was obtained 18 days after the start of therapy in the preoperative group and on Day 28 in the preradiation group: the test was performed after one course of DDP and bleomycin and three to four doses of VDS.

DDP (3 mg/kg or 120 mg/m$^2$, whichever was less) was given with prehydration and a mannitol-induced diuresis as previously described, as was bleomycin (10 units/m$^2$) in a loading dose and (10 units/m$^2$) as a 4-day infusion (6,8). VDS (3 mg/m$^2$) was given as a rapid iv injection through the side arm of a freely flowing iv line. There was no attenuation schedule for either DDP or bleomycin; full doses were given unless the creatinine clearance was < 60 ml/minute or the PFT ($D_LCO$ or vital capacity) had decreased by > 25% of the pretreatment value. The VDS attenuation schedule was to give 100% of the drug dose if the wbc count was ≥ 3000/mm$^3$ and the platelet count was ≥ 100,000/mm$^3$, with only mild neuropathy. Fifty percent of the dose was given if the wbc count was ≥ 2000 but < 3000/mm$^3$, if the platelet count was ≥ 75,000 but < 100,000/mm$^3$, or if there was moderate neuropathy. If the wbc count was < 2000/mm$^3$, if the platelet count was < 75,000/mm$^3$, or if there was severe neuropathy, VDS therapy was withheld pending improvement.

Complete remission (CR) was defined as 100% resolution of all measurable disease for a minimum of 1 month. In patients whose only measurable disease was the primary lesion seen by barium esophagram, a CR was defined as both a normal barium esophagram and endoscopic confirmation (including negative cytology) or pathologic review of the resected specimen indicating that no viable tumor remained. Patients in whom the repeat esophagram was normal, but repeat endoscopy or pathologic review of the resected specimen revealed residual gross or microscopic disease, were classified as partial responders. A partial response (PR) was defined as a ≥ 50% but < 100% decrease in the sum of the product of the two longest perpendicular diameters of measurable masses or a > 50% shrinkage of the tumor as seen on the repeat barium esophagram, for at least 1 month. A minor regression was a ≥ 25% but < 50% decrease in the sum of the two longest perpendicular diameters or definite improve-

TABLE 3.—Site of indicator lesion, response, and response duration

| Patient No. | Indicator site | Response | Response duration (mos) |
|---|---|---|---|
| 1 | Esophagus | PR* | CM† |
| 2 | Esophagus, lymph node | PR‡ | 16+§ |
| 3 | Esophagus | PR | CM |
| 4 | Esophagus | PR | CM |
| 5 | Esophagus | PR | CM |
| 6 | Lymph node | PR | 7 |
| 7 | Esophagus | PR | CM |
| 8 | Esophagus | PR | CM |
| 9 | Esophagus, liver, lymph node | PR | 11+ |
| 10 | Esophagus | PR | CM |
| 11 | Esophagus, lymph node | PR | 6+ |
| 12 | Esophagus | PR | CM |
| 13 | Esophagus | PR | CM |
| 14 | Esophagus | PR | CM |
| 15 | Esophagus | PR¶ | 4+ |
| 16 | Esophagus, lymph node | PR | 4+ |
| 17 | Esophagus | PR | CM |
| 18 | Esophagus, lymph node | PR* | 3+ |
| 19 | Esophagus | PR | CM |
| 20 | Esophagus | PR | CM |
| 21 | Esophagus | PR | CM |

*Normal esophagram, no repeat endoscopy performed.

†CM = combined modality; response duration cannot be ascribed to chemotherapy alone.

‡Normal esophagram, repeat endoscopy; mucosal irregularity, positive biopsy.

§Severe VDS neuropathy and moderate DDP nephrotoxicity led to stopping chemotherapy at 6 mos; radiation therapy was given to primary and right supraclavicular nodes; now without evidence of disease.

¶Initial CM; normal esophagram, esophagoscopy, but positive celiac nodes (laparotomy only). Maintained on chemotherapy alone.

ment in the barium esophagram, with < 50% tumor shrinkage. All responding patients had an improvement in swallowing function.

A curative resection was defined as one in which the primary tumor was removed, the margins of resection were not involved, and the tumor was not adherent to any major structures (aorta or trachea); only regional lymph nodes, which were entirely removed, had tumor involvement. The resection was classified as palliative if the primary tumor and all gross disease were entirely removed but the margins of resection were involved with microscopic tumor, if the tumor was adherent to a major structure and clear margins of resection could not be determined, or if celiac or gastric lymph nodes, which were entirely removed, were involved; the patient had to be grossly free of disease at the end of the operative procedure.

Informed consent was obtained from all patients prior to entrance in the study.

## RESULTS

Of the 46 patients entered in the study to date, 40 are currently evaluable, three are inevaluable for response (two of whom are evaluable for toxicity), and three are pending evaluation. Responses and sites of evaluation are shown in table 3. Of the 40 evaluable patients, 21 (52%) have had PR, and four (10%) have had minor regressions. Fifteen of 26 patients with limited disease had PRs and six of 14 patients with metastases had PRs. Of the 21 patients who had PRs, two patients with metastatic disease and one with disease confined to the esophagus and regional nodes (who received radiation after chemotherapy) had barium esophagrams that re-

turned to normal, as well as disappearance of biopsy-proven malignant lymphadenopathy and would usually be classified as complete responders. However, endoscopy was not performed in two patients and revealed residual microscopic disease in the other. Resected specimens from three of the patients undergoing surgery also showed only microscopic disease.

The durations of response in patients with metastatic disease are 16+, 11+, 7, 6+, 4+, and 3+ months, with one patient having stable disease for 3 months. One patient with metastatic disease relapsed 7 months after starting therapy; he died 6 weeks later. The other five patients with PR remain in remission. One of these patients developed severe peripheral neuropathy and moderate renal insufficiency after 6 months of DVB therapy, and as she still had no evidence of disease outside the primary site and a metastatic supraclavicular lymph node (both of which were in excellent, almost complete remission), definitive radiation therapy (5500 rads) was given to these sites. She is alive without evidence of disease 16+ months from the start of DVB; all therapy has been discontinued.

Of the 26 patients in the combined-modality program, 15 have had PR after a single course of DVB, as seen by barium esophagram. Of the 19 patients undergoing surgery, 14 have had resectable disease (74%); nine of these resections were with curative intent, and five were for palliation. There was one death associated with surgery (5%). One patient who had undergone a palliative resection died of renal failure in the postoperative period; she developed gram-negative pneumonia shortly after her second course of DDP and received gentamicin. She is considered to have had a drug-related death. Except for these two patients, all patients who had resection (curative or palliative) are alive without evidence of disease at 2+ to 14+ months (median followup, 6+).

### Toxicity

Myelosuppression and nephrotoxicity were the major toxic effects. Leukopenia was prominent, with a median wbc count nadir of 1900/$mm^3$ (range, 0.1–6.8 $\times$ $10^3$). Wbc count nadirs were almost invariably seen on Days 8 and 36, following completion of the bleomycin infusion. Ten patients had wbc counts $< 1000/mm^3$; sepsis requiring antibiotic therapy occurred in three patients during this period. One patient, with far-advanced recurrent cervical esophageal carcinoma (PS of 30) and continuous aspiration, died of sepsis. The other patients had uneventful recoveries. Thrombocytopenia was much less frequent (median platelet count nadir, 198,000/$mm^3$; range, 11–234 $\times$ $10^3$).

Nephrotoxicity (peak serum creatinine $\geq$ 2.5 mg/ml) was seen in 19% of the patients but was usually self-limited and returned toward or to pretreatment values. However, one patient, described above, died of renal failure after receiving gentamicin shortly after her second course of DDP.

Most of the patients had alopecia.

Nausea and vomiting was seen in approximately 50% of the patients. Most of those patients who had little or no nausea had received metoclopramide during a phase I–II trial of that antiemetic (9). Diarrhea was seen more often than with DDP and bleomycin alone, again most frequently in those receiving metoclopramide.

Two patients have had clinically significant ototoxicity requiring interruption of DDP therapy. Pulmonary toxicity has not yet been observed.

## DISCUSSION

The current study has demonstrated that DVB is effective in the treatment of esophageal carcinoma, inducing PRs in 52% of the patients (95% confidence limits, 37%–67%). At least four of these responses might previously have been classified as CRs. Our earlier studies with the two-drug combination of DDP and bleomycin or with VDS alone gave CR and PR rates of approximately 20%. The markedly improved response rate with the three drugs used in combination suggests more than just an additive effect. The toxic effects seen with DVB (nephrotoxicity, alopecia, nausea, myelosuppression, and neuropathy) are, in general, not severe and allow use of essentially full doses of all three agents. However, because leukopenia on Days 8 and 36 was significant in a large number of patients and caused us to withhold or attenuate the scheduled VDS dose, we are now limiting the bleomycin infusion to three days (total dose, 40 units/$m^2$), a 20% decrease. This should allow use of the scheduled VDS dose on Days 8 and 36 in a substantially greater percentage of patients.

We have previously accepted in our esophageal protocols patients with a poor PS (< 40 on the Karnofsky scale). However, of the patients with poor PS entered in the DDP and bleomycin, VDS, and DVB protocols, only one (a patient treated with DVB in the current study) had a clinically beneficial response of substantial duration. In addition, these patients are more likely to have serious toxic effects. Similar observations have been made in patients with other solid tumors. Patients with a poor

PS probably should not be considered as candidates for combination regimens involving these agents.

The initial study of DDP and bleomycin in combined-modality therapy suggested that the resection rate of patients receiving preoperative chemotherapy was superior to that seen in a historical control group of patients receiving preoperative radiation therapy (76% vs 54%, respectively) ($P < 0.04$) (10). The mortality associated with surgery was similar (11% vs 12%, respectively). However, although the resection rate was significantly improved, thus affording better palliation by allowing restoration of swallowing function in a greater percentage of patients, long-term survival rates were not increased. We felt that the failure to improve survival was a reflection of the insufficient antitumor activity of the two-drug combination. The current response rate with DVB is approximately 2.5 times that seen with DDP and bleomycin; the number of patients with near CR is also substantially greater. Whether the increased activity of DVB will improve the survival rate of patients with potentially operable lesions is not yet known. The extremely poor prognosis with the conventional therapy of surgery and/or radiation for even these patients, with relatively early disease, suggests that progress in this field will require the development of effective combined-modality techniques. The current program is hopefully an example of such an approach. In addition to continued study in esophageal carcinoma, similar studies in patients with epidermoid carcinoma of the head and neck, a tumor in which DDP and bleomycin have also been shown to be active, should also be considered.

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# Treatment of Advanced Squamous Cell Carcinoma of the Head and Neck With Cisplatin, Bleomycin, and Methotrexate (PBM)[1,2]

**Thomas J. Ervin,* Ralph Weichselbaum, Daniel Miller, Michael Meshad, Marshall Posner, and Richard Fabian[3]**

## SUMMARY

**Twenty-nine of 32 patients with advanced squamous cell carcinoma of the head and neck were treated with two courses of an intensive chemotherapy regimen consisting of cisplatin, bleomycin, and methotrexate (PBM). The frequency of toxic effects (in 29 evaluable patients) associated with this regimen was low. Serious neutropenia occurred in 17% and thrombocytopenia in 10% of the courses. Nausea (50%) and severe vomiting (6%) were easily managed. All 26 (100%) evaluable patients with squamous cell carcinoma had significant reduction (> 50%) of measurable tumor. Complete regression was noted in seven patients (27%). Fifteen patients were treated with PBM prior to receiving radiation therapy or surgery as definitive treatment for their disease. Antitumor response in these patients was rapid, occurring within 3 weeks from the initiation of chemotherapy. Maximal antitumor effect was achieved by Week 6 of induction chemotherapy. No patient showed tumor regrowth prior to the institution of radiation therapy or surgery. Twelve patients with recurrent disease following previous radiotherapy and/or surgery also received PBM chemotherapy. In our study, previous radiotherapy or surgery did not diminish the antitumor effect of PBM, but toxicity was more severe.**

[Cancer Treat Rep 65:787–791, 1981]

Recently, there has been an increased interest in the usefulness of chemotherapy for patients with advanced squamous cell carcinoma of the head and neck. Despite the lack of clear-cut superiority of combination chemotherapy when compared with single-agent chemotherapy in randomized trials (1,2), combination chemotherapy has supplanted single-agent therapy as standard chemotherapy for these neoplasms (3–5). The antitumor effect of combination chemotherapy is most evident in patients treated prior to definitive radiation therapy and/or surgery (6–9).

Chemotherapy used as induction therapy prior to radiation therapy and/or surgery is given with a dual purpose. First, the cytoreduction of regional disease prior to radiation therapy and surgery should increase the efficacy of these local treatment modalities. In a recent analysis of patients receiving single-agent high-dose methotrexate (MTX) prior to radiation therapy and/or surgery, patients having significant response to induction MTX had an increased 2-year disease-free survival compared to those patients who did not respond to induction therapy (10). Second, in addition to preoperative cytoreduction, the administration of chemotherapy prior to local treatment identifies the active antitumor agents which may provide adjuvant treatment for microscopic metastases outside the local treatment field. This application is of particular importance since there is as yet no evidence for effectiveness of adjuvant therapy in squamous cell carcinoma of the head and neck.

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[3]Sidney Farber Cancer Institute, Boston, MA.

**Reprint requests to:* Thomas J. Ervin, MD, Sidney Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

In this paper, we report on the early findings of induction combination chemotherapy using cisplatin, bleomycin, and MTX (PBM) in 16 previously untreated patients with advanced stage III and IV carcinoma of the head and neck. Additionally, the results of this regimen in 13 patients failing previous radiation therapy and surgery are reported.

## MATERIALS AND METHODS

From October 1, 1979, to April 1, 1980, 32 patients with advanced carcinoma of the head and neck were entered in our protocol (table 1). Twenty-nine of the 32 patients were evaluable for toxicity; the three remaining patients never received chemotherapy. Twenty-six patients were evaluable for response. Of the three patients excluded from response evaluation, one with the histologic diagnosis of diffuse histiocytic lymphoma had a complete response to one cycle of PBM chemotherapy, and two with adenocarcinoma had no response to PBM chemotherapy.

The characteristics of the 29 evaluable patients are noted in table 2. Thirteen patients had been treated with previous surgery and/or irradiation, whereas 16 patients received their chemotherapy as induction chemotherapy prior to definitive treatment. All patients entered in this protocol were evaluated at the Multidisciplinary Head and Neck Cancer Clinic at the Sidney Farber Cancer Institute, all were seen simultaneously by the three participating physicians and the physical examination reviewed, and all were staged according to the American Joint Committee for Cancer Staging system (11).

All patients receiving chemotherapy in this protocol were hospitalized at the Sidney Farber Cancer Institute, Children's Hospital Medical Center, or the Beth Israel Hospital (Boston, MA). In all patients chemotherapy was administered by one of the investigators (T. J. Ervin). The treatment protocol is outlined in figure 1. Patients were hospitalized from Day 1 through Day 7 of the first course of chemotherapy. Cisplatin at a dose of 20 mg/m²/day was begun on Day 1 and was continued through Day 5 of the course of chemotherapy; it was given as a 2-hour infusion following a 1500-ml normal saline prehydration. Patients received additional post-treatment hydration. To avoid excess nausea, all patients received iv antiemetics. Bleomycin given at a dose of 10 units/m²/day as a continuous infusion was begun on Day 3 after nausea had subsided, and was continued through Day 7 of the course of chemotherapy. After discontinuation of the bleomycin chemotherapy, patients were discharged from the hospital, usually not receiving any medication. Patients then returned to the outpatient clinic for subsequent chemotherapy, receiving MTX at a dose of 200 mg/m² iv on Days 15 and 22 of the chemotherapy course to avoid possible crossover nephrotoxicity with cisplatin. Leucovorin rescue was given 24 hours after each dose of iv MTX. The leucovorin was given at a dose of 20 mg orally every 6 hours for 48 hours. All patients were evaluated for response during the last few days of each course of chemotherapy. If there was evidence of tumor regression at 28 days from the start of chemotherapy, the PBM protocol was begun on Day 29 and was continued to Day 56, for a total of 8 weeks from the start of all chemotherapy. At the end of the second course of PBM chemotherapy, all patients were evaluated for response.

TABLE 1.—Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Patients entered | 32 |
| Patients evaluated | 29 |
| Exclusion from protocol | |
| Lymphoma | 1* |
| Adenocarcinoma | 2† |

*The patient, subsequently diagnosed as having diffuse histiocytic lymphoma, had complete response to 1 cycle of chemotherapy.

†Patients had no response to PBM chemotherapy.

TABLE 2.—Characteristics of evaluable patients

| Characteristic | No. of patients |
|---|---|
| Total | 29 |
| Previous treatment | |
| Radiation therapy + surgery | 12 |
| Radiation therapy | 1 |
| No previous treatment | 16 |
| Age in yrs | |
| Median: 55 | |
| Range: 9–77 | |
| Sex | |
| Males | 23 |
| Females | 6 |
| Stage | |
| III | 2 |
| IV | 27 |

## RESULTS

The toxicity of the PBM protocol is outlined in tables 3 and 4. Nausea was the most common toxic effect noted, occurring in 50% of the treatment courses. In most patients, the nausea was limited to the first 3–5 days of hospitalization and was easily

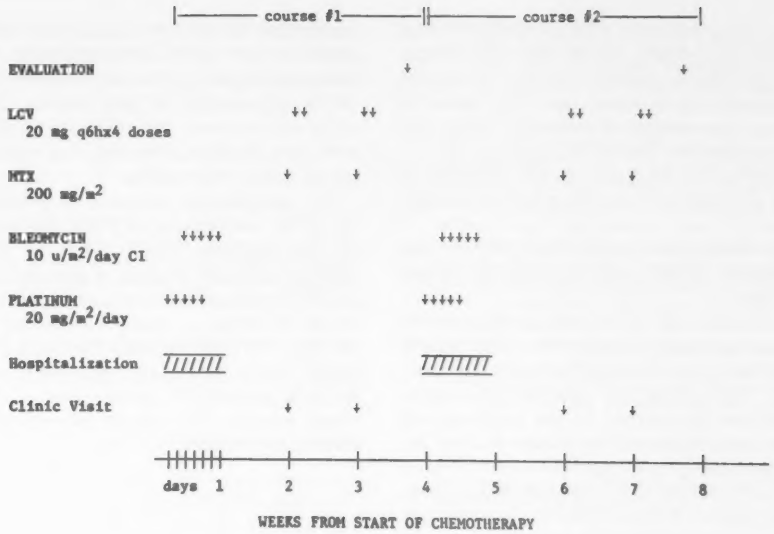


FIGURE 1.—PBM chemotherapy scheme. LCV = leucovorin; CI = continuous infusion.

managed with oral or iv antiemetics. Weight loss averaged < 2 kg over the 8-week chemotherapy period. Patients having received previous therapy were more debilitated at the time of chemotherapy and this group lost approximately 3 kg/two PBM courses. Conversely, the patients having received no previous treatment lost < 1 kg during the 8-week induction chemotherapy. Vomiting was well-controlled with the antiemetic program used. Mild nephrotoxicity was seen in 11% of the patients. Ototoxicity was not quantitated, but grossly impaired auditory acuity was not noted in our series. In each case, the decrease in creatinine clearance was not serious enough to withhold further chemotherapy and often resolved before the next drug was to be given. Hematologic suppression was also unusual in our series. Neutropenia occurred in ap-

TABLE 3.—Toxicity of PBM chemotherapy

| Toxic effect | % of courses |
|---|---|
| Nausea | |
| Grade 1–2 | 50 |
| Grade 3–4 | 4 |
| Vomiting | |
| Grade 1–2 | 4 |
| Grade 3–4 | 2 |
| Nephrotoxicity | |
| Creatinine clearance | |
| 25%–50% decrease | 11 |
| > 50% decrease | 0 |
| Hematologic toxicity | |
| Wbc count (cells/mm$^3$) | |
| 1000–2000 | 9 |
| < 1000 | 8* |
| Platelet count (cells/mm$^3$) | |
| 50,000–100,000 | 4 |
| < 50,000 | 6 |

*1 death from sepsis in a previously treated patient.

TABLE 4.—Additional toxicity associated with PBM chemotherapy (53 evaluable courses)

| Toxic effect | No. of courses |
|---|---|
| Early death, unrelated to chemotherapy | 2 |
| Skin rash (bleomycin) | 3 |
| Pulmonary toxicity (bleomycin) | 2* |
| Fever (bleomycin) | 2 |
| Fever (cisplatin) | 1 |
| Fluid imbalance | 3 |
| Mucositis (grade 3) | 2 |
| Infections | |
| Aspiration pneumonia | 1 |
| Sepsis (death) | 1 |
| Delay in surgery (neutropenia) | |
| < 1 wk | 1 |
| 1–2 wks | 1 |

*1 mild, 1 moderate reaction.

proximately 17% of patients, with 8% of the courses associated with potentially life-threatening neutropenia. One previously treated patient developed sepsis at an outlying hospital and died from an overwhelming gram-negative infection after the cessation of antibiotics. Thrombocytopenia was infrequent in our series, with no patients manifesting hemorrhagic diathesis or requiring platelet transfusions.

Overall, the incidence of major toxic effects was no more common in the second treatment course than in the first.

Other associated toxic effects (table 4) were unusual and were medically manageable. One patient did manifest moderate bleomycin-induced pulmonary toxicity. This patient has gradually improved with the addition of steroids to the regimen and currently has only minimal symptoms during exertion. Two previously untreated patients had their planned surgery delayed due to neutropenia following the second course of chemotherapy. In each case, the neutropenia resolved rapidly, and the surgery, as planned, was accomplished without complication. As is usual with protocols using high-dose cisplatin chemotherapy, potassium, magnesium, and calcium wasting were noted. Patients regularly required potassium and magnesium replacement during the 5 days of cisplatin chemotherapy.

The response to PBM chemotherapy is reported in table 5. Responses were defined as: *complete response*—the disappearance of all clinically or radiographically measurable tumor; *partial response*—a > 50% reduction in the product of the greatest perpendicular diameters of the lesions; and *disease progression*—a > 25% enlargement in the product of the greatest perpendicular diameters of the lesions or the appearance of new lesions. If there was a disparity between the response at different tumor sites, the smallest response was taken as the measure of tumor regression.

TABLE 5.—Response to PBM chemotherapy

| | No. of evaluable patients responding | |
|---|---|---|
| Total | | |
| > 50% partial response | 19 | (73%) |
| Complete response | 7 | (27%) |
| | 26 | |
| Previously treated patients | | |
| > 50% partial response | 8* | |
| Complete response † | 3 | |
| | 11 | |
| Previously untreated patients | | |
| > 50% partial response | 11 | |
| Complete response | 4 | |
| | 15 | |

*Duration (mos): 3+, 4+, 4, 6+, 6, 2+, 6+, 4.

†Patients with complete response received a subsequent radiotherapy boost of 2500–5000 rad.

For purposes of analysis, all responses reported are after two courses of PBM chemotherapy (table 5). The response rate is 100% for the 26 patients treated with two courses of chemotherapy. The duration of response for previously treated patients is noted in table 5, with the median duration > 4 months. Three previously treated patients have relapsed following the two courses of chemotherapy. In each patient the relapse was at the site of previous disease. No patient has relapsed with new distant metastases.

## DISCUSSION

In this paper we report the short-term followup on a highly successful combination regimen consisting of high-dose cisplatin, continuous-infusion bleomycin, and intermediate-dose MTX with oral leucovorin rescue. The overall response rate of 100% is superior to the results of others using a similar treatment regimen (1,2,7,8,12–14). In our series, previous radiotherapy or surgery did not alter the response to PBM chemotherapy.

The efficacy of high-dose cisplatin, bleomycin, and MTX with leucovorin rescue suggests that the dose and timing of treatment are important for these agents. Specifically, continuous-infusion bleomycin may enhance cytotoxicity, either by its interaction with cisplatin-induced sublethal damage or by its own enhanced cytotoxicity as compared to bolus bleomycin (15). MTX given at a dose of 200 mg/m$^2$ ensures therapeutic serum MTX concentrations and avoids rapid excretion of the drug as a cause of treatment failure.

PBM chemotherapy is a highly efficacious combination that deserves further trial in previously untreated patients or selected recurrent patients for whom a potentially curative local treatment is still feasible. Response to treatment is rapid and well-tolerated in these patients and subsequent radiotherapy and surgery are not compromised by PBM chemotherapy.

For patients with recurrent squamous cell carcinoma of the head and neck, PBM chemotherapy is efficacious in producing antitumor response. However, potential cumulative drug toxic effects,

such as nephrotoxicity, ototoxicity, and anorexia from cisplatin, or pulmonary fibrosis related to bleomycin administration, will limit its long-term use in these patients.

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# Tamoxifen-Induced Regression of Cerebral Metastases in Breast Carcinoma[1]

**Robert W. Carey,* James M. Davis, and Nicholas T. Zervas[2]**

Treatment of intracranial metastases is one of the most difficult aspects of breast cancer management. In the past, patients have generally been treated with corticosteroids and whole-brain irradiation after angiographic or nuclear scan documentation of intracranial metastases in association with clinical manifestations such as signs of increased intracranial pressure, focal neurologic signs, or seizure disorders. In radiosensitive tumors like metastatic breast cancer, such management results in transient palliation, with a median response duration of 3–6 months (1). In patients with solitary lesions, little evidence of disease elsewhere, and long disease-free intervals, surgical extirpation is occasionally quite successful. In general, there is little expectation that systemic chemotherapy is effective, presumably because of failure to cross the blood-brain barrier. Nitrosoureas, effective in glioblastomas, do cross the blood-brain barrier, presumably due to lipid solubility, but have not been demonstrated to be of major value in metastatic breast cancer. In the present case, remarkable regression of multiple cerebral metastases documented by computerized cranial tomography (CCT) followed treatment with tamoxifen in an estrogen receptor-positive patient who also experienced regression of multiple soft tissue metastases.

## CASE REPORT

A 43-year-old woman presented with a laterally placed breast lesion adjacent to a scar where benign fibrocystic disease had previously been excised. She underwent a left radical mastectomy on June 2, 1969, which revealed adenocarcinoma of the left breast with seven of ten axillary lymph nodes positive. After surgery, the patient was well until January 15, 1976, when physical examination revealed signs of right parietal lobe dysfunction with weakness and poor coordination on the left side. A contrast-enhanced CCT on February 7, 1976, showed numerous nonenhancing, low-density, rounded, 6–18-mm lesions in both brain hemispheres. A carotid angiogram confirmed the presence of mass lesions at this time. In March 1976 the patient was treated with 2400 rads to the whole brain for palliation of metastatic carcinoma.

On November 18, 1976, she was noted to have a 3.5 × 3.5-cm left submandibular lesion, a 2.5 × 2.5-cm left axillary lesion biopsy of which showed recurrent adenocarcinoma. She was started on chemotherapy with 150 mg of CCNU every 6 weeks and 50 mg of procarbazine orally three times a day for 10 days. Subsequently, on March 10, 1977, treatment was changed to 50 mg of cyclophosphamide twice a day for 14 days, 40 mg of prednisone orally for 14 days, 500 mg of 5-FU on Days 1 and 8, and 50 mg of methotrexate iv on Days 1 and 8. This program was continued throughout early 1977, and by October 28, 1977, all skin and lymph node metastatic lesions were noted to have increased in size. Biopsy of a subcutaneous nodule on December 8, 1977, showed a positive estrogen receptor at 47.5 femtomols/mg of protein.

On March 22, 1978, the patient was referred to Massachusetts General Hospital for evaluation because of progressive enlargement of multiple brain lesions on consecutive CCT scans plus progression of soft tissue metastases. At this time CCT showed three discrete lesions: two in the left temporal lobe and one in the left cerebellum (fig 1A). Also present were a 1 × 1-cm right perianal lesion, two breast nodules measuring 2 × 2 and 3 × 2.5 cm, and a 1.5 × 1.5-cm left supraclavicular lymph node. Despite the abnormal CCT scan the patient was neurologically normal.

The patient was started on 10 mg of tamoxifen twice a day. By May 8, 1978, the breast nodules had improved. One lesion had decreased from 2.2 × 2.5 to 2.4 × 2.0 cm. A second nodule had decreased from 2 × 2 to 1.7 × 1.7 cm. The supraclavicular mass had decreased from 1.5 × 1.5 to 1.2 × 1.2 cm. CCT at this time showed marked regression of the brain lesions (fig 1B). As of September 5, 1978, with continuation of tamoxifen, both of the breast nod-

[1]Received Sept 22, 1980; revised Dec 29, 1980; accepted Jan 15, 1981.

[2]Medical Oncology Unit, Massachusetts General Hospital, Boston.

**Reprint requests to:* Robert W. Carey, MD, Medical Oncology Unit, Massachusetts General Hospital, The Cox Building, 100 Blossom St, Boston, MA 02114.

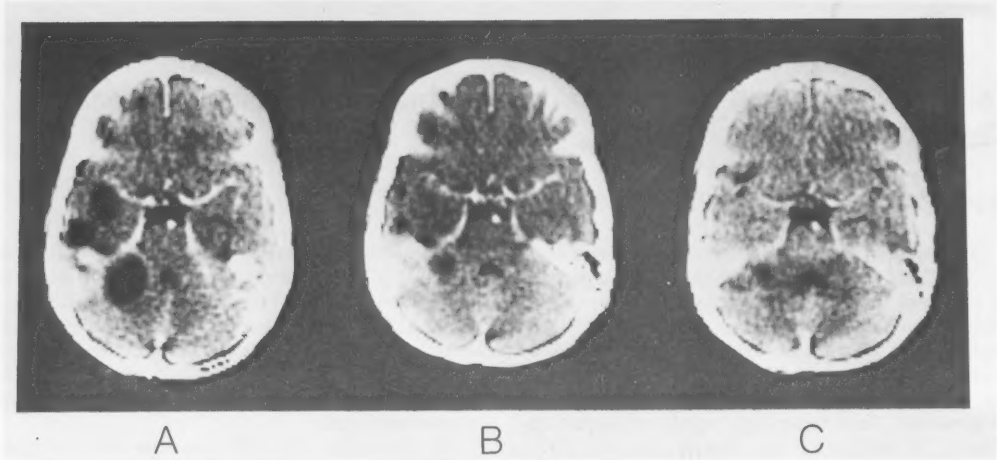


FIGURE 1.—Serial contrast-enhanced CCT scans at same level showing progressive diminution in size of multiple lesions in the temporal lobe and cerebellum. A) March 24, 1978; B) May 4, 1978; and C) September 5, 1978.

ules measured < 1 cm in diameter, the perianal metastasis had disappeared, and the right supraclavicular mass had disappeared. CCT (fig 1C) showed no lesion in the left temporal lobe and nearly total disappearance of the lesion in the left cerebellum. The last CCT scan done June 17, 1980, was totally normal. Therapeutic intervention with tamoxifen appeared to result in regression of the multiple large soft tissue and presumed cerebral metastases. The patient had been receiving 10 mg of prednisone daily since March 1977, and this continued during the period of tamoxifen therapy until withdrawal was begun in September 1978. Subsequently, prednisone was withdrawn and the patient has continued to do well. All the soft tissue metastases have regressed completely, and the patient has now been neurologically stable for 30 months. A malignant cytologically positive pericardial effusion noted on July 29, 1979, responded to a single dose of intrapericardial nitrogen mustard and has not reaccumulated.

## DISCUSSION

The chance of 1-year survival after demonstration of cerebral metastases is between 3% and 20% (1), and it is noteworthy that this patient had already survived 2 years before radiologically demonstrable disease progression occurred. There is little evidence to suggest that chemotherapy effective in systemic metastases has significant benefit in cerebral metastatic disease. Part of the problem in evaluation lies in heretofore inadequate methods of assessing results of treatment. The advent of CCT represents a significant advance. This noninvasive modality allows determination of chronologic change in the morphology of metastatic brain neoplasms and their response to therapeutic measures. CCT literature includes reports of the response of primary brain tumors to radiation and/or chemotherapy (2–5) but is scant in the evaluation of the response of metastatic lesions (6–8). We are unaware of any reports of metastases that have "disappeared" following therapy. Diminished contrast enhancement of neoplasms has been reported to occur after administration of steroids (6), but in our case little or no contrast enhancement was present before steroids were utilized, and the steroid dose had been stable for 1 year. Steroids are thought to cross the blood-brain barrier and clearly are effective on a short-term basis in 60%–75% of patients with intracerebral metastases (1). Usually the benefit is thought to be associated with reduction of edema around tumors rather than a direct antitumor effect. In the present case, there was no edema associated with the lesions. The CCT picture and clinical course were thought to exclude one other entity, eg, porencephalic cyst, which was considered in initial interpretation of the CCT images.

The lack of efficacy of systemic agents against intracerebral metastases in general is probably due to primary ineffectiveness of the systemic agents rather than the blood-brain barrier (9).

In this case, the agent leading to clinical regression of peripheral and intracranial tumors was tamoxifen, the estrogen antagonist demonstrated to

compete with estrogen for binding to the cytosol estrogen receptor found in most hormone-responsive breast cancer (10). Since the initial reports of Cole et al (11) in 1971, tamoxifen is well-established as being clinically effective in about 50% of patients with metastatic breast cancer. The finding of a positive estrogen receptor elevates the response rate in breast cancer patients to > 60%, while responses in estrogen receptor-negative patients are rare (12).

As noted, we believe the simultaneous regression of CCT-documented cerebral lesions and measurable multiple soft tissue metastases in this patient validates the efficacy of tamoxifen in bringing about an impressive clinical result in a patient whose brain lesions had progressed after radiation therapy and while receiving chemotherapy and low-dose corticosteroids. Tamoxifen may be similarly effective in other estrogen receptor-positive patients who develop intracranial metastases.

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# Reversible Brachial Plexopathy Following Primary Radiation Therapy for Breast Cancer [1]

**Andrew L. Salner,[2,*] Leslie E. Botnick,[2] Andrew G. Herzog,[3] Michael A. Goldstein,[3] Jay R. Harris,[2] Martin B. Levene,[2] and Samuel Hellman [2]**

## SUMMARY

**Reversible brachial plexopathy has occurred in very low incidence in patients with breast carcinoma treated definitively with radiation therapy. Of 565 patients treated between January 1968 and December 1979 with moderate doses of supervoltage radiation therapy (average axillary dose of 5000 rad in 5 weeks), eight patients (1.4%) developed the characteristic symptoms at a median time of 4.5 months after radiation therapy. This syndrome consists of paresthesias in all patients, with weakness and pain less commonly seen. The symptom complex differs from other previously described brachial plexus syndromes, including paralytic brachial neuritis, radiation-induced injury, and carcinoma. A possible relationship to adjuvant chemotherapy exists, though the etiology is not well-understood. The cases described demonstrate temporal clustering. Resolution is always seen.**

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A difficult and not infrequent problem in patients with cancer is the differential diagnosis of neuropathy. Many well-recognized types of brachial plexus dysfunction are seen in patients with malignant disease. These include radiation-induced injury, tumor infiltration, paralytic brachial neuritis, and dysfunction associated with postmastectomy edema. Radiation-induced injury is seen after high doses of radiation therapy to the axilla after mastectomy or to the region of the brachial plexus for the treatment of a variety of tumors (1,2). Infiltration of the plexus may occur with many tumors but is most commonly seen with breast cancer, lung cancer, or lymphoma (3–5). Paralytic brachial neuritis, an acute syndrome of severe pain followed by weakness, is seen in patients with lymphoma undergoing therapy and in patients without malignant disease (6,7). Dysfunction of the plexus has also been noted in patients with postmastectomy edema, presumably due to compression of nerves by lymphedema (8).

Since its inception in 1968, the Joint Center for Radiation Therapy has taken great interest in the local treatment of breast cancer with primary radiation therapy without mastectomy. During the course of this experience, a previously unrecognized entity, reversible brachial plexopathy, has been observed in a small number of patients so treated. This syndrome clearly differs from the others in its clinical and laboratory characteristics and to date has always been reversible.

## METHODS

Between January 1968 and December 1979, 565 patients with breast carcinoma were treated definitively with radiation therapy, without mastectomy, at the Joint Center for Radiation Therapy. Seventeen of these patients were treated for bilateral disease; thus, a total of 582 breasts were treated.

[1]Received Oct 6, 1980; revised Feb 17, 1981; accepted Mar 3, 1981.

[2]Joint Center for Radiation Therapy, Department of Radiation Therapy, Harvard Medical School, Boston, MA.

[3]Department of Neurology (*A. G. Herzog*), and Division of Medical Oncology, Department of Medicine (*M. A. Goldstein*), Beth Israel Hospital, Boston, MA.

**Reprint requests to*: Dr. Andrew Salner, Joint Center for Radiation Therapy, Harvard Medical School, 50 Binney St, Boston, MA 02115.

All stages of disease were represented in this patient population (161 had stage I, 251 had stage II, and 170 had stage III), though the preponderance of patients had stage I and II disease.

The technique of therapy has evolved somewhat over the 12-year period. Most patients (all in recent years) have undergone excisional biopsy of the primary lesion. In addition, in the past several years, many patients, particularly those in the premenopausal or perimenopausal groups, have undergone diagnostic low axillary sampling to determine nodal pathology (9). Lymphoscintigraphy was carried out to assess location of the internal mammary lymph nodes (10). External-beam irradiation utilizing a 4-MeV linear accelerator was then given to tangential fields encompassing the breast and internal mammary nodes. Also, an anterior supraclavicular-axillary field was treated, with dose prescribed to a depth of 5 cm, to a total dose of 4400–4600 rad. In patients with positive axillary lymph nodes, a posterior axillary boost field was added, with dose prescribed to the same point as the anterior field (depth = separation − 5 cm) for an additional 400–1000 rad. Estimated dose to the brachial plexus was 100% ± 5% of the prescribed dose. No bolus was used, and the hanging block technique was implemented in recent years to prevent matchline complications. Charts and portal films were reviewed in detail, and no patient with reversible brachial plexopathy treated prior to use of this technique had evidence of a matchline overdose. Usual doses have been 4500–5000 rad to the breast and nodal areas in 4½ to 5½ weeks, at 200 rad/day. This external beam treatment has been followed in 2 weeks by iridium strand implantation in most patients, delivering an additional 2000–2200 rad to the original tumor volume. This technique has been well-described elsewhere, as have results of cosmesis, local control, complications, and survival (11–14).

## RESULTS

Eleven patients have exhibited symptoms of brachial plexus dysfunction. The symptoms of three of these were of readily apparent etiology. One patient received a 6700-rad axillary dose for very advanced disease and subsequently developed symptoms presumed secondary to radiation-induced injury. Two other patients had active inflammatory/orthopedic disease of the shoulder and the cervical spine prior to irradiation, and their symptoms did not significantly change with followup. The remaining eight patients with reversible brachial plexopathy are described in table 1. The median age was 43 years, and all disease stages were represented. Four of the eight patients underwent diagnostic low axillary sampling. The median axillary dose was 5000 rad. Seven of eight patients received adjuvant chemotherapy, though only six had symptoms that began after their drug program started. A median time of 3 months existed between the end of radiation and the beginning of chemotherapy, and a median interval of 4.5 months was noted between the end of radiation therapy and the onset of symptoms. Of the six patients whose chemotherapy started prior to symptoms, five received cyclophosphamide, methotrexate, and 5-FU (CMF) and one received methotrexate and 5-FU (MF). These drugs were given in the usual monthly schedule (15). In four patients, more soft tissue reaction was seen than normally noted with this regimen of moderate irradiation. No difference in symptoms could be ascertained as compared with those patients without such reactions.

The symptoms of these eight patients may be characterized as follows. The initial presentation consisted of hand and forearm paresthesias in all patients, mild to moderate shoulder and axilla pain in four, and mild finger weakness in two. Subse-

TABLE 1.—Patients with reversible brachial plexopathy

| Patient No. | Age (yrs) | Clinical stage | Results of axillary sampling | Brachial plexus dose (rad) | Radiation-drug interval (mos) | Chemotherapy | Radiation-symptom onset interval (mos) |
|---|---|---|---|---|---|---|---|
| 1 | 49 | I ($T_1N_0M_0$) | — | 5000 | — | — | 12 |
| 2 | 47 | III ($T_3N_0M_0$) | — | 4600 | 0 | CMF | 14 |
| 3 | 48 | I ($T_1N_0M_0$) | — | 5000 | 5 | Melphalan | 1.5 |
| 4 | 43 | II ($T_2N_1M_0$) | Positive | 5150 | 2.5 | CMF | 10 |
| 5 | 27 | III ($T_3N_1M_0$) | Negative | 5000 | 1.5 | MF | 2 |
| 6 | 29 | III ($T_3N_1M_0$) | — | 5200 | 0.5 | CMF | 5 |
| 7 | 29 | III ($T_2N_2M_0$) | Positive | 5000 | 0 | CMF | 4 |
| 8 | 43 | II ($T_2N_1M_0$) | Positive | 5400 | 2 | CMF | 3 |
| Median | 43 | | | 5000 | 3 | | 4.5 |

TABLE 2.—Relationship between adjuvant chemotherapy and reversible brachial plexopathy

| | Total No. of patients | No. of patients with— | |
|---|---|---|---|
| | | No plexopathy | Plexopathy |
| No adjuvant chemotherapy before symptoms | 440 | 438 | 2 (0.4%) |
| Adjuvant chemotherapy before symptoms | 125 | 119 | 6 (4.8%) |

quently, the paresthesias were stable for 3–6 months and then improved. Patients with pain noted improvement in this symptom over 2–3 months. Weakness developed in three additional patients over the ensuing 6 months, thereby having affected five of the eight patients. Two of these patients had severe weakness producing almost complete paralysis, flaccidity, and atrophy of the involved arm. All regained full strength. In five of the eight patients, complete resolution of all symptoms was seen. In the remaining three patients, minimal residual paresthesias persisted. No symptoms were seen in the contralateral arm or shoulder.

Nerve conduction studies and electromyograms were obtained for six of the eight patients. These have revealed slowing of nerve conduction velocity across the thoracic outlet, which has shown marked improvement in the four patients who have undergone serial studies. Also, slowing of nerve conduction velocity was seen across the area of the ulnar groove and carpal tunnel in three of the six patients studied, and mild decrease in total number of motor units has been seen in two. The significance of these findings is not known. Studies of irradiated patients without brachial plexus symptoms are currently under way to ascertain whether similar nerve conduction abnormalities exist.

As demonstrated in table 2, six of the eight patients with reversible brachial plexopathy received adjuvant chemotherapy prior to the onset of symptoms. This represents an incidence of 4.8% (six of 125 patients) in patients who have received definitive breast irradiation and adjuvant drugs. Only two patients developed reversible brachial plexopathy with no adjuvant chemotherapy prior to onset of symptoms, representing an incidence of 0.4% (two of 440 patients). This difference in incidence is statistically significant ($P < 0.001$; $X^2 = 13.16$).

As seen in figure 1, there was a temporal clus-

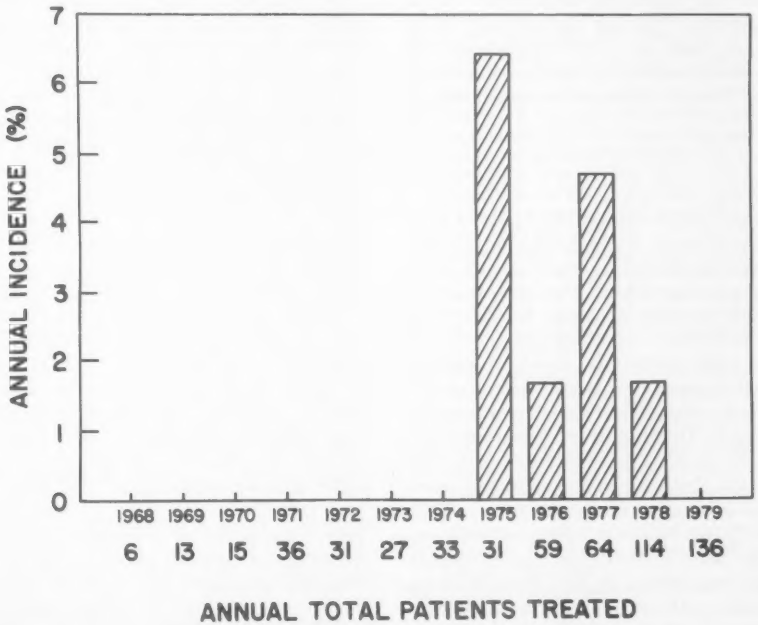


FIGURE 1.—Annual incidence of reversible brachial plexopathy.

TABLE 3.—Characteristics of brachial plexus neuropathies

| | Paralytic brachial neuritis | Classic radiation-induced injury | Carcinoma | Reversible brachial plexopathy |
|---|---|---|---|---|
| Onset | With treatment | 6–24 mos after radiation; < 6000 rad, > 12 mos | 24–36 mos | Median: 4.5 mos |
| Pain | Severe, disappears with weakness | Mild to severe, persistent | Severe, persistent | Occasional, mild, disappears |
| Weakness | Severe, reversible | Moderate, progressive | Moderate, progressive | Mild to severe, occasional, reversible |
| Paresthesias | Occasional, mild | Moderate | Mild | Always, mild and reversible |
| Dose relation | | Yes | | No |
| Soft tissue reaction | No | Yes | | Occasional |
| Spontaneous resolution | Usually | No | No | Yes |

tering of these eight cases between September 1975 and February 1978. No new cases have appeared since that time, despite an increasing number of patients being treated and an increasing number receiving adjuvant chemotherapy.

Detailed review of port films, therapy records, and calculations did not reveal significant deviation from the planned treatment. Chest roentgenograms at the time of symptom onset and thereafter were carefully reviewed in attempts to rule out recurrent disease. No change in overall technique could account for either the beginning or the end of the syndrome's incidence. Though the number of affected patients is small, no relationship was seen between reversible brachial plexopathy and axillary sampling, stage, dose, or subsequent local or distant control.

## DISCUSSION

Many abnormalities of the brachial plexus may be seen in cancer patients. Though each represents a spectrum of clinical symptoms, they can be characterized as outlined in table 3. Paralytic brachial neuritis, or acute brachial neuritis, has been observed in patients with or without neoplastic disease (6,16). Patients with lymphoma, either Hodgkin's disease or non-Hodgkin's lymphoma, seem predisposed to this entity, particularly during treatment with either radiation therapy or chemotherapy (6). Paralytic brachial neuritis is characterized by severe shoulder pain that disappears with the onset of weakness and atrophy of arm musculature. This process frequently involves both upper extremities, may involve the lower extremities, and is usually self-limited and reversible. It has been well-described in patients without malignancy in heredofamilial settings (17), after serum immunization or vaccination (18,19), after viral illness (20), in an epidemic setting frequently involving a military camp (21), or after an antiallergy desensitizing injection (22). Because this entity has been seen after antigenic stimuli, after a viral illness, in an epidemic setting, or in an immunocompromised host, immune hypersensitivity, allergic, and infectious etiologies have been proposed.

Paralytic brachial neuritis differs in many ways from the reversible brachial plexopathy described in this report. The former begins during treatment while the onset of the latter is seen several months after treatment. Pain, an important feature of paralytic brachial neuritis, is not prominent in reversible brachial plexopathy. Weakness, also always present in paralytic brachial neuritis, is variable in reversible brachial plexopathy. Paralytic brachial neuritis usually involves muscles proximal to the plexus, ie, scapular and rhomboid muscles. This phenomenon has not been observed in patients with reversible brachial plexopathy. Electromyograms and nerve conduction studies reveal two quite different patterns: general demyelinization through any segment in paralytic brachial neuritis, but distinct segment slowing in reversible brachial plexopathy.

Classic radiation-induced injury of the brachial plexus has been typically seen after large doses of radiation to the axilla (12). This is presumably due to either direct injury or compression of nerves by fibrosis. There is a time-dose complication relationship, with larger doses (> 6000 rad) having an increased incidence of plexus damage and a shorter interval to symptom onset (23). With doses < 6000 rad, radiation-induced injury rarely occurs in < 1 year, as opposed to reversible brachial plexopathy which almost always occurs within several months. Pain and weakness, which may be mild to moderate and progressive with radiation-induced injury, are occasional and always reversible with reversible brachial plexopathy.

Numerous reports in the literature have discussed differential diagnosis between radiation-induced injury and carcinomatous involvement of the plexus (24–26). The latter, secondary to breast cancer in most instances, is responsive to radiation therapy or other cytotoxic treatment (3–5). Its typical onset, 2–3 years after initial treatment, is delayed as compared with reversible brachial plexopathy. Pain is very prominent and persistent, as compared with the occasional, mild, and reversible pain of reversible brachial plexopathy. Similarly, weakness is moderate and progressive in carcinomatous involvement as opposed to the reversible, occasional involvement in reversible brachial plexopathy. Brachial plexus symptoms have also been associated with postmastectomy edema, either with or without postoperative radiation therapy (8). This entity presumably is due to compression of nerves by edema.

No obvious etiology of reversible brachial plexopathy is evident. Edema was not observed by either patient or physician. Nerve compression secondary to fibrosis of soft tissues did not explain this syndrome in which most patients had no evidence of soft tissue reaction at the time of onset of symptoms. The temporal clustering suggests an infectious etiology, and the possible relationship to adjuvant chemotherapy suggests either an infectious or immune etiology. If an infectious etiology were to be considered, a common organism, usually not pathogenic, with low infectivity, may be postulated as the agent. Radiation therapy, and particularly its combination with chemotherapy, may have caused expression of this organism as a neurotropic pathogen. Clustering could be explained by the presence of this organism for an isolated time. Attempts at identifying a point source were unsuccessful, particularly since many of these patients were treated in different divisions in our department. No common physician, technologist, chemotherapist, source of drugs, etc could be identified to explain the temporal clustering. Also, no preceding viral illness could be identified, and no patients subsequently suffered from herpes zoster or other illnesses suggesting immune compromise. Though numerous explanations may be offered, or even a combination thereof, a definitive etiology cannot be determined at this time. Careful neurologic evaluation of all treated patients has continued and has failed to reveal any new cases of reversible brachial plexopathy for 30 months despite treatment of 230 patients, all of whom have subsequently been followed for at least 6 months.

In conclusion, reversible brachial plexopathy, a newly recognized entity, has been observed in very low incidence (1.4%) in patients with breast cancer treated definitively with radiation therapy. The characteristic symptoms differ from other defined brachial plexus neuropathies. A possible relationship to adjuvant chemotherapy exists, with an increased incidence in those patients treated with adjuvant drugs. Spontaneous resolution, usually complete, is always seen.

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# Intra-arterial BCNU Chemotherapy for the Treatment of Malignant Gliomas of the Central Nervous System: A Preliminary Report[1,2]

**Harry S. Greenberg,* William D. Ensminger, Joachim F. Seeger, Glenn W. Kindt, William F. Chandler, Kathleen Doan, and Shaker R. Dakhil[3]**

## SUMMARY

**We treated six patients with unilateral malignant astrocytomas with pulse doses of intra-arterial BCNU (200 mg/m² initially, with escalating doses every 6–8 weeks) via transfemoral selective internal carotid catheterization. Four patients had had partial resections without prior radiation therapy and received no steroids. They had decreased tumor stain and surrounding edema after two to four cycles of chemotherapy. For one patient, results of the neurologic examination returned to normal, with total disappearance of her tumor as assessed by computerized tomographic scan. Objective tumor response continued for 7 months in three patients and for 3 months in one. In two of the four patients, chemotherapy was discontinued because of retinal toxicity and not because of treatment failure. Two patients had had partial resections and radiation therapy. One patient had stable disease for 4 months, and the second had progressive disease with gradual visual loss beginning in the infused eye 3 weeks after the second treatment. The catheterization procedure is safe; it was without immediate complication in 17 BCNU infusions. In summary, high-dose intra-arterial BCNU is well-tolerated and is an effective initial chemotherapeutic modality.**

[Cancer Treat Rep 65:803–810, 1981]

Grade III and IV malignant astrocytomas (glioblastoma multiforme) are uniformly rapidly fatal with current medical treatment. The best results in histologically proven malignant astrocytomas with radiation therapy and systemic chemotherapy have extended the median survival to only 1 year (1–3). The localized nature of this tumor and its relatively isolated arterial supply, with the majority of tumors having one carotid artery as the main supply, make intra-arterial chemotherapy attractive. In a recent experimental study, Crafts et al (4) demonstrated that intracarotid administration of BCNU achieved fourfold higher drug levels in the brain than those attained by iv administration of the same dose. Thus, a higher peak tumor concentration could be achieved with less systemic toxicity.

Based on the above observations, we have initiated a clinical trial using escalating doses of BCNU given by short (12–15-minute) intracarotid arterial infusion in patients with grade III and IV malignant astrocytomas. Although the trial is not complete, we feel the results in the first six patients have been so satisfactory that a preliminary report is warranted.

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[3]Department of Neurology (*H. S. Greenberg*), Division of Hematology/Oncology, Department of Internal Medicine, Upjohn Center for Pharmacology (*W. D. Ensminger, K. Doan,* and *S. R. Dakhil*), Division of Neuroradiology, Department of Radiology (*J. F. Seeger*), and Division of Neurosurgery, Department of Surgery (*G. W. Kindt* and *W. F. Chandler*), University of Michigan Medical School, Ann Arbor.

**Reprint requests to*: Harry S. Greenberg, MD, Department of Neurology, University of Michigan Medical School, Ann Arbor, MI 48109.

## MATERIALS AND METHODS

Six patients with histologically proven grade III and IV malignant astrocytomas, who were referred to the University of Michigan Hospital between December 1979 and April 1980, were entered in this study. The four patients without prior radiation therapy were consecutive and unselected. Eligibility requirements included the ability to tolerate arterial catheterization, an estimated life expectancy of 2 months, and an initial Karnofsky rating $\geq 30$. All patients were evaluated initially by a neurologist (H. S. Greenberg) and an oncologist (W. D. Ensminger) and underwent a pretreatment computerized tomographic (CT) scan. The corticosteroid dexamethasone was administered when clinically indicated, with the dose adjusted at weekly intervals. Diphenylhydantoin, carbamazepine, and phenobarbital were used as anticonvulsants. Written informed consent was obtained from all patients.

### Drug Delivery System

Patients were treated with BCNU administered through a selective internal carotid artery catheter placed percutaneously using the femoral approach. Immediately prior to infusion and within 10 minutes of catheter placement BCNU was dissolved in ethanol (100 mg of BCNU/2 ml of ethanol) and was then added to 125 ml of normal saline. This BCNU-containing solution was then infused continuously over 12–15 minutes by an IMED 922 (Medical Electronics Corp) infusion pump at a rate of 600 ml/hour.

### Treatment and Followup Evaluation

A neurologic examination, functional performance rating, chemical profile, and cbc were obtained before treatment and every 2 weeks during treatment. The initial BCNU dose in each patient was 200 mg/m$^2$ of body surface area. The dose was escalated by 50 mg/m$^2$ with each succeeding treatment if there was no significant myelosuppression (ie, wbc count nadir $< 3000/mm^3$; platelet count nadir $< 100,000/mm^3$) or other toxicity. Treatments were repeated every 6–8 weeks. A CT scan, cbc, chemical profile, and chest radiogram were obtained prior to each treatment. An angiogram was obtained at the time of treatment. Patients were premedicated im with morphine, secobarbital, and thiethylperazine, and were given morphine iv during the drug infusion as required. Naloxone was used to reverse the morphine at the end of the drug infusion.

Whole-brain radiation therapy in conventional doses was to be administered to patients who were not previously given such therapy and in whom, at the completion of any course of chemotherapy with intra-arterial BCNU, progressive disease was observed (*see* below). Whole-brain radiation therapy was also to be given to diminish potential cumulative systemic toxicity, if, at the completion of chemotherapy with intra-arterial BCNU to a total dose of 1500 mg/m$^2$, there was evidence of intracranial tumor by neurologic examination or CT scan. Two patients have required radiotherapy for progressive disease and no patient has reached the 1500-mg/m$^2$ cut-off for cumulative dose.

### Determination of Response

A complete response was defined as the resolution of all abnormalities as assessed by neurologic examination and CT scan, without steroid medication. A partial response was defined as a decrease but less than the total disappearance of the mass lesion as assessed by CT scan, plus a stable or improved neurologic examination, while steroid medication was either decreased or unchanged. Stable disease was defined as no change in the size of the tumor as assessed by CT scan, without significant change in the neurologic examination, while doses of steroids were held constant. Progressive disease was defined as an increase in the size of the mass as assessed by CT scan, and/or progressive worsening of neurologic signs and symptoms directly attributable to the tumor.

## RESULTS

We have treated six patients with unilateral malignant astrocytomas of the brain with pulse doses of intra-arterial BCNU (200 mg/m$^2$ initially, and then in incremental doses every 6–8 weeks) via transfemoral selective internal carotid catheterization (table 1).

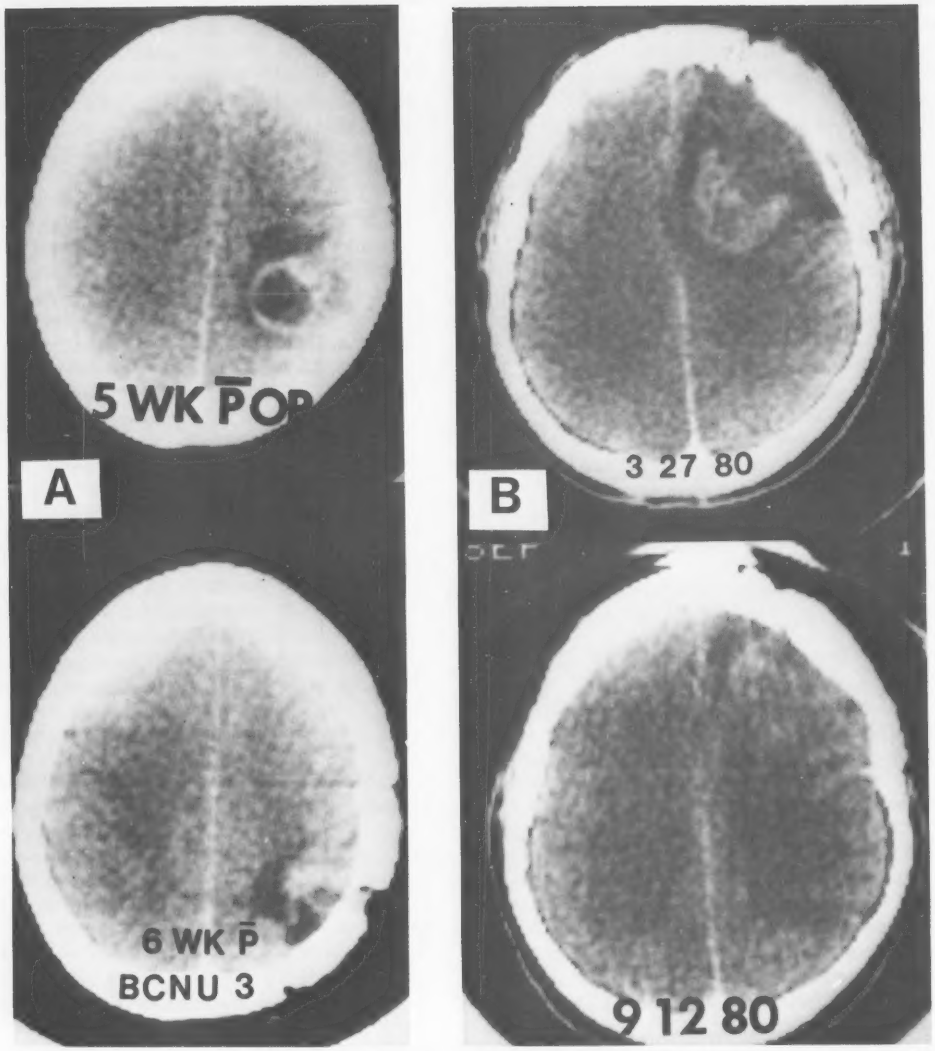
Four of the treated patients had had partial resections of malignant astrocytomas without prior radiation therapy and received no steroid medication. All four patients had significant tumor bulk following surgery. After two to four cycles of chemotherapy, three of these patients demonstrated decrease in tumor size (partial response), with decreased tumor stain and surrounding edema on contrast-enhanced (infusional) CT scan (fig 1). A fourth patient had a complete response, with a normal neurologic examination and total disappearance of tumor as assessed by CT scan (fig 2).

Objective tumor response lasted 7 months in two of these patients (Nos. 1 and 3). Following radiation therapy, they are alive at 13 and 9 months, respectively; the former has a moderate expressive aphasia and the latter has a mild left hemiparesis. Objective

TABLE 1.—Initial response to intra-arterial carotid BCNU chemotherapy

| Patient No. | Tumor location | BCNU courses (doses, mg/m²) | Steroids (after last course) | Clinical response | CT scan response | Complications |
|---|---|---|---|---|---|---|
| 1 | Left frontal | 200, 250, 300, 250* | None | Improved, normal examination | Complete | Ipsilateral facial dermatitis (reversible) |
| 2 | Right parietal | 200, 250, 300*,† | None | Improved, normal examination | Partial | Right amaurosis with vision returning to 20/40 |
| 3 | Right parietal | 200, 250, 300* | None | Improved, normal examination | Partial | Right fundal hemorrhages and exudates |
| 4 | Right temporal | 200, 250*,† | None | Improved, normal examination | Partial | Right amaurosis |
| 5‡ | Left frontal | 200, 250* | Yes | Disease progression | Disease progression | Left amaurosis |
| 6‡ | Right frontal | 200, 250, 300 | Yes | Stable | Stable | None |

*Course of chemotherapy after which side effect developed.
† Chemotherapy terminated because of retinal toxicity, not because of response failure.
‡ Radiation therapy given prior to chemotherapy.


5 WK P
A
6 WK P
BCNU 3
3 27 80
B
9 12 80

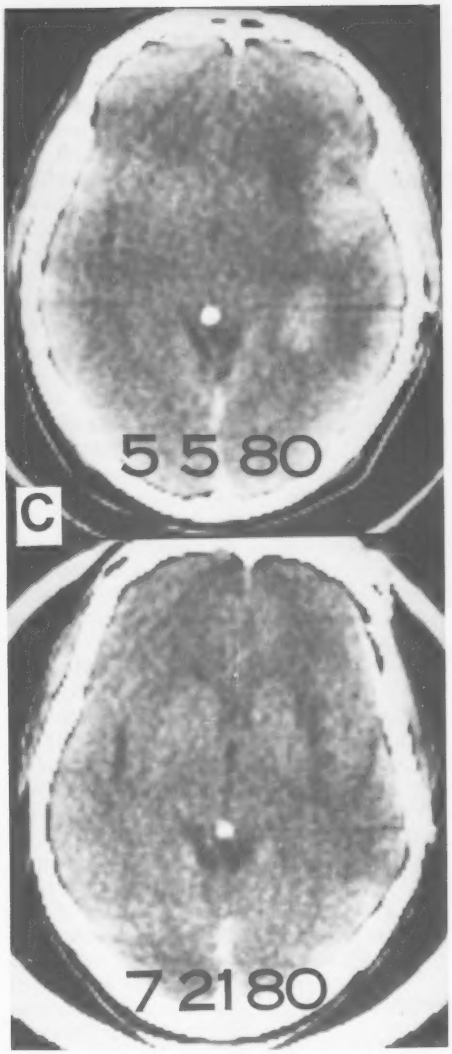



FIGURE 1.—Pretreatment and posttreatment CT scans through the area of maximum tumor size in partial responders to intra-arterial BCNU.

**A:** A 56-yr-old right-handed female (patient 2) with a right parietal malignant astrocytoma 5 wks following subtotal resection (top) and 6 wks following the 3rd course of intra-arterial BCNU, with decreased ring enhancement and size of tumor (bottom).

**B:** A 35-yr-old right-handed white male (patient 3) with a right parietal malignant astrocytoma 1 wk following subtotal resection (top) and 6 wks following the 3rd course of intra-arterial BCNU, with marked decrease in tumor enhancement and size, accompanied by resolution of edema and 3rd ventricular shift (bottom).

**C:** A 56-yr-old right-handed white male (patient 4) with a right parietal temporal malignant astrocytoma 3 wks following subtotal resection (top) and 6 wks following the 2nd course of intra-arterial BCNU, with marked decrease in tumor enhancement and size, accompanied by resolution of edema (bottom).

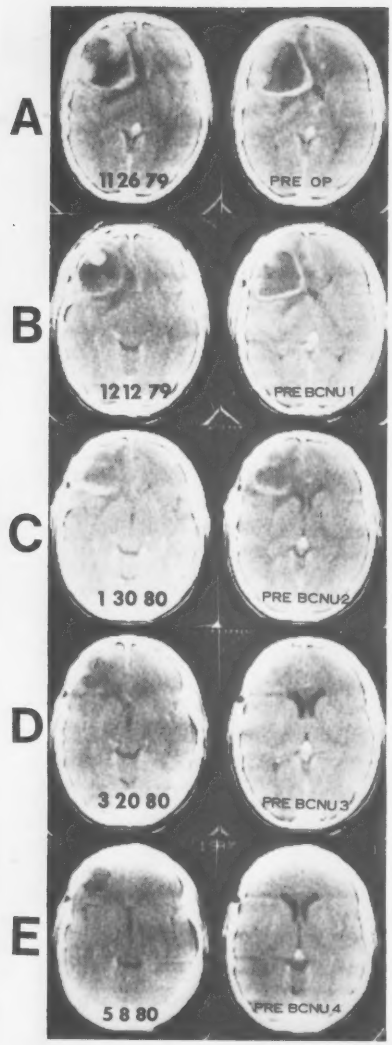



FIGURE 2.—A 50-yr-old female with a grade III malignant astrocytoma who had a partial resection on November 30, 1979, and started receiving intra-arterial BCNU chemotherapy, at a dose of 200 mg/m$^2$, on December 12, 1979. Her neurologic examination after surgery revealed literal paraphasic dyslexic reading errors and mild right hyperflexia. Her preoperative CT scan (A) and serial scans obtained prior to 4 successive courses of intra-arterial BCNU (B-E) show progressive tumor regression with no evidence of neoplasm on May 8, 1980 (E). On June 25, 1980, her neurologic examination was normal and the CT scan was unchanged from May 8, 1980.

tumor response was ongoing in two other patients (Nos. 2 and 4) at 7 and 3 months, respectively, when retinal toxicity required termination of therapy. They are alive after radiation therapy at 12 and 8 months, respectively. All four patients now require continuous steroid administration to minimize their neurologic symptoms (table 2).

Two patients (Nos. 5 and 6), who had had partial resection and radiation therapy with rapid progression of their tumors, have received intra-arterial BCNU therapy with concurrent steroid administration. One of these patients had stable disease for 4 months, as assessed by CT scan and clinical examination, and lived for 5 months. The other patient had progressive disease by neurologic examination and CT scan, and died at 3 months (tables 1 and 2).

The significant toxic effects in four of the six patients have been unilateral fundal hemorrhages and exudates, progressing to blindness in three patients. All four patients developed these symptoms 3 weeks following their second (patients 4 and 5) or third (patients 2 and 3) courses of chemotherapy. In three patients, the visual loss progressed inexorably to blindness over 3 days. One patient (No. 2) had her vision return to 20/40 in the involved eye. The fourth patient (No. 3) has had visual blurring with normal acuity due to a scotoma produced by a retinal hemorrhage. Two patients had fluoroscein angiograms showing an arterial phase leak consistent with a toxic retinal vasculitis. The ophthalmic artery has been normal, with no evidence of a generalized vasculitis on repeat internal carotid artery angiograms.

The catheterization procedure has been safe, with no immediate complications in 17 infusions of BCNU. During the infusion, all patients felt moderately severe pain in the ipsilateral eye, and were nauseous for 3–6 hours following the infusion. All patients were able to be discharged on the day following treatment. Mild blood cell count depression was usually seen 4–6 weeks after BCNU infusion. After one infusion only, there was a wbc count nadir $< 2500/mm^3$ or a platelet count nadir $< 75{,}000/mm^3$; the counts were 1000 and 23,000, respectively. No patients have required transfusions, antibiotics, or hospitalization for toxicity.

## DISCUSSION

BCNU is a non-phase-specific alkylating agent and appears to be the most active single drug in brain tumor chemotherapy (1–3). Preclinical studies suggest that for BCNU and alkylating agents in general, high peak concentrations are more important than prolonged low-level exposure for cytotoxic effects (5). Computer modeling studies by Fenstermacher and Cowles (6) indicated that an appropriate agent with a short serum half-life could be used to generate both higher peak levels and an increased exposure by the internal carotid route. In vivo monkey studies with BCNU have shown a 5-minute serum half-life, and a fourfold higher brain concentration when the same concentration of drug is administered by an intra-arterial rather than by an iv route (4).

Intracarotid arterial BCNU has been used by Avellanosa et al (7) for gliomas and by West et al (8–10) for cerebral metastases at a dose of 100 mg/$m^2$ every 3–4 weeks by bolus infusions. There were eight evaluable patients with malignant astrocytomas who had received various previous treatments. Four patients had definite tumor response with decreased edema and enhancement, two relapsing after 3–4 months. Two patients had stable disease, and two had relentless disease progression.

Hochberg et al (11) treated nine patients with recurrent glioblastoma (after surgery and irradiation) with single high-dose iv BCNU (600-1400 mg/$m^2$) followed by autologous bone marrow rescue. Six of nine patients without previous BCNU exposure had clinical improvement or disease stabilization, with CT scan improvement lasting $> 6$ months in five patients. The response at a dose of 600–1400 mg/$m^2$ of iv BCNU suggests a steep dose-response curve requiring a higher dose of BCNU than used by West and co-workers (8).

In our study, using higher doses than previously reported, four patients without previous treatment have had definite tumor shrinkage and decreased edema. The shrinkage of their tumors can only be attributed to BCNU chemotherapy. The one patient with a complete response for 4 months had not received steroids since the fifth postoperative day, 7 days before her first course of intra-arterial chemotherapy. To our knowledge, this is the first report of intra-arterial or iv chemotherapy alone producing complete disappearance of a malignant astrocytoma as assessed by clinical and CT evaluation. Our preliminary results suggest that many malignant astrocytomas may be sensitive to BCNU chemotherapy if the concentration reaching the tumor is sufficient.

The response in the two patients previously treated with radiation therapy has been less impressive, with one patient having stable disease for 4 months, and the other patient having evidence of progressive disease. In these patients, radiation therapy may have selected out clones of cells that would have been responsive to chemotherapy. Cells that have grown back may be more resistant and therefore less likely to respond to any form of therapy (12).

TABLE 2.—Duration of response in intra-arterial carotid BCNU chemotherapy

| Patient No. | Courses of treatment | Duration of chemotherapy response (mos) | Current clinical status following radiation therapy |
|---|---|---|---|
| 1 | 4 | 7 | Alive at 13 mos, expressive dysphasia |
| 2 | 3 | 7* | Alive at 12 mos, left hemiparesis |
| 3 | 3 | 7 | Alive at 9 mos, left hemiparesis |
| 4 | 2 | 3* | Alive at 8 mos, left hemiparesis |
| 5† | 2 | None | Death after 3 mos |
| 6† | 3 | 4 | Death after 5 mos |

*Chemotherapy terminated because of retinal toxicity, not because of response failure.
†Radiation therapy prior to chemotherapy.

The progressive visual loss experienced by three patients with hemorrhagic retinopathy may have been due to a retinal vasculitis, as suggested from an experiment in dogs by DeWys and Fowler (13). Although there was no angiographic evidence of a generalized cerebral vasculitis, the fluorescein angiogram in two patients showed an arterial phase leak consistent with a toxic retinal vasculitis. This complication has not been reported by Avellanosa et al (7), West et al (8), Madajewicz et al (9), and Yamada et al (10) using doses of BCNU of 100 mg/m$^2$ for up to four treatments, but has been seen in both eyes in patients receiving high-dose iv BCNU (800 mg/m$^2$) with bone marrow rescue.[4]

In summary, malignant astrocytomas can be treated with the chemotherapeutic agent BCNU given by the intra-arterial route. If relapse occurs after effective initial response, radiation therapy can be used. The duration of response has been 7 months in three patients who have then responded or stabilized with radiation therapy. The major chronic toxic effect appears to be ipsilateral visual loss secondary to a toxic retinal vasculitis, prompting termination of treatment in two patients.

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[4]Shingleton BS, Gorder JR, Biengang DC, et al (Massachusetts Eye and Ear Infirmary) to Greenberg HS. Personal communication, Sept 24, 1980.

# Risk of Brain Metastasis From Small Cell Carcinoma of the Lung Related to Length of Survival and Prophylactic Irradiation [1,2,3]

Ritsuko Komaki,* James D. Cox, and William Whitson [4,5]

## SUMMARY

**The problem of brain metastasis from small cell carcinoma (SCC) of the lung has been appreciated for many years, but the magnitude of the problem has been underestimated. Recent studies have shown that the risk of brain metastasis increases as survival is prolonged. Although prophylactic cranial irradiation (PCI) has reduced the frequency of brain metastases, the effect on risk estimates of differences in the periods of observation was not evaluated. From 1974 through 1979, 131 patients with SCC of the lung who had no evidence of brain metastasis by radionuclide or computerized tomographic scan were treated in the Division of Therapeutic Radiology at the Medical College of Wisconsin Affiliated Hospitals. PCI was started in 1977; 57 patients received it and 74 did not. To correct for the differing periods of observation for the two groups, an actuarial calculation of the probability of brain metastasis was used. The calculated rate of clinical failure in the brain for patients who did not receive PCI was 28% at 12 months and 58% at 24 months. The calculated failure rate in the brain for patients who received PCI was 11% at 12 and 24 months. The difference in the probability of brain metastasis between the patients who did or did not receive PCI is highly significant ($P < 0.01$). The true benefit of PCI becomes apparent only when the risk of intracranial metastasis is evaluated by methods that correct for incomplete followup. PCI eliminates the progressive increase in the risk of brain metastasis that accompanies increased survival and is important to maximize the probability for cure of patients with SCC.**

[Cancer Treat Rep 65:811–814, 1981]

The problem of brain metastasis in patients with cancer of the lung has been appreciated for many years (1–3), and the potential for prophylactic irradiation of sites of predilection for microscopic disease has been noted (4). In spite of treatment with systemic chemotherapy, the clinical diagnosis of brain metastasis has been made in 22% of patients with small cell carcinoma (SCC) (5). Autopsy studies revealed that 40%–45% of patients who die from SCC have intracranial metastases (5,6). It may be reasoned, however, that the risk of metastasis is low and the response to irradiation for overt metastasis is sufficiently high, so that prophylactic cranial irradiation (PCI) benefits few patients.

The studies of Nugent et al (7) showed that in the absence of PCI, the probability of brain metastasis increased with lengthening survival of patients with SCC. They found that the actuarial probability of brain metastasis rose to 80% for patients who lived ≥ 2 years after the diagnosis of SCC. Studies at the Medical College of Wisconsin showed that

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[3]Presented at the Second World Conference on Lung Cancer, Copenhagen, Denmark, June 9–13, 1980.

[4]Division of Therapeutic Radiology, Medical College of Wisconsin Affiliated Hospitals, Milwaukee.

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*_Reprint requests to:_ Ritsuko Komaki, MD, Division of Therapeutic Radiology, Medical College of Wisconsin Affiliated Hospitals, 8700 W Wisconsin Ave, Milwaukee, WI 53226.

TABLE 1.—Characteristics of patients studied

| Characteristic* | No PCI | PCI |
|---|---|---|
| No. of patients | 74 | 57 |
| Male:female | 58:16 | 39:18 |
| Age (yrs) | | |
| Range | 42–81 | 40–81 |
| Median | 59 | 60 |
| Limited disease:extensive disease | 37:37 | 28:29 |
| Chest irradiation | 34 (46%) | 3 (5%) |
| Chemotherapy | 1 (1%) | 23 (40%) |
| Chest irradiation and chemotherapy | 39 (53%) | 31 (54%) |

*Unless specified otherwise, values = No. of patients.

one half of patients with overt brain metastases from SCC died from progressive intracranial disease (8).

If PCI actually eliminates microscopic metastases that would become manifest, it is justifiable to use PCI systematically with the expectation that a very large proportion of patients will benefit. However, there has not been any other study to corroborate the very high rate of intracranial failure noted by Nugent et al (7), and PCI has not been assessed as a function of the duration of survival. The following study was undertaken to evaluate the risk of brain metastasis in patients with SCC who did or did not receive PCI.

## PATIENTS AND METHODS

This review included patients entered in studies at the Medical College of Wisconsin between 1974 and 1979. Previous reports of these studies documented the patient characteristics, treatments, and results, including response rates, local control rates, median survival, and survival beyond median with the various regimens used (9–12). The patients evaluated had a performance status $\geq 50$ and had no clinical evidence of brain metastasis. In addition, radionuclide or computerized tomographic brain scans were done on all patients and were found to be negative.

The studies at the Medical College of Wisconsin did not include PCI until 1977. Since that time, all patients have received prophylactic treatment. As a result, there were 74 evaluable patients who had not received PCI and 57 who had undergone PCI. The groups were quite similar in regard to sexual composition, age, and ratio of patients with limited disease to those with extensive disease (table 1).

In the earliest years of this study, patients with limited disease only received thoracic irradiation. Combination chemotherapy, primarily with cyclophosphamide, doxorubicin, and vincristine, was used consistently since 1976. Since patients who did or did not receive PCI occupy two different time periods, a comparison is not valid unless there is a correction for duration of survival. Similar to the studies of Nugent et al (7), the actuarial or life-table method was used to evaluate the probability of brain metastasis. This method corrects for differences in survival that might have resulted from differing approaches to treatment of the extracranial disease.

Patients who received PCI were treated with 4–25-mev X-rays or with $^{60}$Co teletherapy. Parallel-opposed, lateral fields were used. Treatment was begun within the first week after diagnosis. Nineteen patients who had PCI received 3000 rad in 2 weeks in the midsagittal plane, and 38 patients received 2500 rad in 2 weeks.

Brain metastasis was evaluated on the basis of clinical data including symptoms, neurologic examination, radionuclide scans, and computerized tomograms. Histologic confirmation was not obtained. At autopsy, no patient had intracranial metastases that were unsuspected clinically.

## RESULTS

At the time of evaluation, two patients in the group that had not received PCI were alive and well (200 and 270 weeks). Seventeen patients in the group that had received PCI were alive and well and nine patients were alive with disease. Their median survival times were 40 and 57 weeks, respectively. The overall median survival of the two treatment groups was the same: 35 weeks for the patients without PCI and 36 weeks for the patients with PCI.

Eighteen of the 74 (24%) patients who did not have PCI developed clinical evidence of brain metastasis. Two patients (4%) who received PCI had

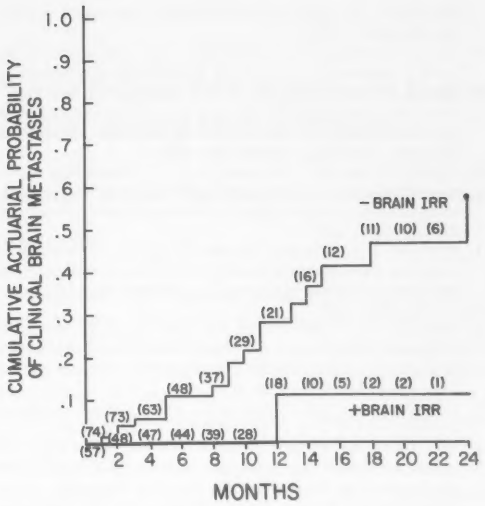


FIGURE 1.—Probability of brain metastasis by treatment group.

intracranial failure. The median intervals from the diagnosis of SCC to the diagnosis of intracranial metastasis were not significantly different between the two groups: 35 weeks in the group without PCI versus 50 weeks in the PCI group. The intervals from metastasis to death were also similar (21 weeks). Approximately one half of the patients who developed brain metastasis actually died of their intracranial disease (eight of 18 patients in the group without PCI versus one of two in the PCI group).

The actuarial probability of clinical failure in the brain increased steadily with increasing survival (fig 1). By 24 months, the probability of clinical brain metastasis had risen to 58% in the patients who had not received PCI. By contrast, the two patients who developed brain metastasis after PCI did so at approximately 1 year, giving an actuarial probability of 11%, and no further failures were seen beyond that time.

## DISCUSSION

The findings in this study corroborate the results of Nugent et al (7). The crude clinical failure rate of 24% (18 of 74 patients) agrees with most reports in the recent literature. This rate represents a major underestimation of the risk of brain metastasis. When the survival of patients with SCC is brief, microscopic metastases may not have sufficient time to become apparent clinically. Patients who live longer are at greater risk for clinical manifestations of intracranial disease. As the effectiveness of chemotherapy has increased, the extracranial disease has been better controlled. Since the drugs, including the nitrosoureas, do not cross the blood-brain barrier in sufficient concentration to eliminate or suppress the microscopic metastases (5), prolonged survival is associated with an increased rate of CNS failure.

The probability of intracranial metastases, calculated on an actuarial or life-table basis, rose to 58% for patients who lived 2 years. This is remarkably similar to the clinical failure rate observed by Nugent et al (7). Their rate of 80% included the 12% of patients who presented with brain metastases and also included autopsy findings. Reduction of the clinical failure rate to 11% for the patients who received PCI gives a much more accurate representation of the importance of prophylactic treatment. This indicates that microscopic metastases can be eliminated even with very modest doses of irradiation, when combined with chemotherapy. Lower doses of irradiation (2000 rad in ten fractions in 2 weeks) did not significantly reduce the frequency of brain metastases (13). The slightly higher dose of 2500 rad in ten fractions in 2 weeks was quite effective. Whether this difference represents a very steep dose-response relationship or a chemotherapy-radiotherapy interaction cannot be determined on the basis of the available data.

Matthews (14) reported the autopsy findings in 91 patients who had been treated with intensive chemotherapy. Of 90 who had examination of the brain, 36 had evidence of metastases to the parenchyma, the pituitary, or the meninges. There were 17 patients from studies of the Radiation Oncology Branch, National Cancer Institute (15) and 20 from studies of the Working Party for Cancer of the Lung who received PCI; eight of these 37 patients (22%) had CNS metastases. Of the 53 patients who did not receive PCI, 28 (53%) had microscopic evidence of CNS metastases. Some of the CNS metastases in the patients who received PCI may have been outside the field of irradiation. It is also possible that reseeding of the CNS developed from recurrent disease in the chest and other sites. However, the significant ($P < 0.01$) reduction in CNS metastases indicates that permanent sterilization of microscopic foci resulted from the PCI.

Although more data based on actuarial or similar calculations that take duration of survival into account will give a clearer understanding of the value of PCI, there is no longer any doubt that PCI is effective in eliminating intracranial metastases. Eventual failure in the brain is so consistent if PCI is omitted from the treatment that PCI should be included in all treatment regimens for SCC. The

effectiveness of the modest dose of 2500 rad in 2 weeks, in combination with chemotherapy, permits a reduction in the short-term morbidity and may even be more important in minimizing long-term morbidity as prolonged survival becomes more frequent.

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# Phase II Trial of Cisplatin in Refractory Childhood Cancer: Children's Cancer Study Group Report[1,2]

**Edward S. Baum, Paul Gaynon, Lowell Greenberg, William Krivit, and Denman Hammond[2,*]**

## SUMMARY

**A total of 114 children with solid tumors refractory to conventional therapy were evaluated for response and/or toxic effects after receiving cisplatin at doses of 3.0–4.5 mg/kg with aggressive hydration and mannitol diuresis every 3 weeks; a minimum of two courses was required for evaluation of response (110 patients). Objective responses were noted in 18 patients: rhabdomyosarcoma (three), Wilms' tumor (three), osteogenic sarcoma (three), Ewing's sarcoma (two), neuroblastoma (one), undifferentiated sarcoma (one), hepatoblastoma (one), ovarian teratoma (one), hepatocellular carcinoma (one), embryonal carcinoma of the mediastinum (one), and thymoma (one). Twenty-six patients had some evidence of renal toxicity. Asymptomatic hearing loss was commonly found when audiometry was performed (eight of 18 patients tested). Eight additional patients had symptomatic hearing problems—tinnitus or hearing loss. Myelosuppression was mild. Hypomagnesemia and/or hypocalcemia were common but only one patient had symptoms. Cisplatin, administered at a dose of 3.0 mg/kg with aggressive hydration and mannitol diuresis, is reasonably well-tolerated. Its role in the therapy for those tumors against which it shows activity remains to be determined.**

[Cancer Treat Rep 65:815–822, 1981]

Cisplatin (DDP) has been used singly and in combination in a number of phase II and III studies (1). The Food and Drug Administration has approved it for treatment of nonseminomatous testicular cancer. DDP is thought to be a bifunctional alkylating agent with preferential binding to guanine. Some evidence of selective inhibition of RNA and protein synthesis has been reported (2). On the other hand,

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**Reprint requests to:* Children's Cancer Study Group, Operations Office, 1721 N Griffin Ave, Los Angeles, CA 90031.

DDP may act by enhancement of the antigenicity of the tumor system (3).

Renal toxicity had been the major dose-limiting factor (4,5). Merrin (6) and others found decreased renal toxicity when DDP was administered over 6–8 hours with aggressive hydration and diuresis. Hayes et al (7), using a similar approach, were able to increase the dose from 1 to 3 mg/kg without observing irreversible renal toxicity.

Others (8–11) have reported on the use of DDP in various childhood neoplasias. A minimum of two objective responses have been reported in neuroblastoma and osteogenic sarcoma. Single responses have been reported in medullary carcinoma of the thyroid and in nongonadal seminoma.

## MATERIALS AND METHODS

### Eligibility and Evaluability

Eligibility requirements for this study included recurrent and progressive tumor resistant to conventional therapy, a measurable lesion, and normal renal function. Patients without bone marrow replacement had absolute neutrophil counts > 1500/mm$^3$ and platelet counts > 75,000/mm$^3$. Patients with only one kidney were not excluded. Appropriate informed consent was obtained from the patients or their parents. No minimum expectation for survival was required.

Patients who underwent adequate pretreatment and posttreatment evaluation of extent of disease and received at least two infusions of DDP were considered to be evaluable for tumor response. Patients who received two or more infusions of DDP but for whom the extent of tumor was not adequately evaluated or patients who received only one infusion of DDP because of toxic effects possibly related to DDP were considered to be evaluable for toxicity only.

### Treatment Plan

Patients were hospitalized for treatment. DDP was administered at a dose of 3 mg/kg with aggressive hydration and diuresis every 3 weeks; there was an optional increase to 4.5 mg/kg after three courses if no response had been seen. Prior to each infusion, urinalysis, serum creatinine, BUN, electrolytes, uric acid, calcium, magnesium, SGOT, bilirubin, cbc and platelet count, creatinine clearance, and weight and blood pressures were obtained. If there was unresolved residual toxicity, the dose was not reduced; instead, therapy was delayed at the discretion of the investigator. Thirty minutes before the start of infusion, an antiemetic was administered. Strict input and output measurements were begun and reviewed hourly. The DDP was protected from light and administered in 1500 ml/m$^2$ of 5% dextrose in water with 4.5 g/liter of NaCl, 250 mg/kg of mannitol, and 1 mEq/kg of KCl over an 8-hour period. After 4 hours, administration of fluid to compensate for any accumulated fluid deficit was begun as 5% dextrose in water with 4.5 g/liter of NaCl and 20 mEq/liter of KCl and was continued over the next 4 hours. The antiemetic was repeated as needed. After 8 hours the infusion was terminated. Urinalysis, serum chemistries, and weight and blood pressure were rechecked. Iv fluids, constituted to correct any electrolyte abnormalities, were administered at a rate of 125 ml/m$^2$/hour for 16 hours. At the end of this time, parenteral fluids were continued until oral fluids were tolerated.

If urine output decreased to < 150 ml/m$^2$/hour during the DDP infusion, 250 mg/kg of mannitol was given as a rapid infusion. If this did not induce a prompt diuresis, furosemide was administered at a dose of 0.5 mg/kg, as an iv push.

### Definition of Tumor Response

For solid tumors, a complete response (CR) was defined as the disappearance of all measurable tumor. A partial response (PR) was defined as a > 50% reduction in the sum of the products of two perpendicular diameters of each measurable lesion, with no lesion increasing in size and no appearance of any new lesion. For acute leukemia, Children's Cancer Study Group (CCSG) criteria were applied.[3]

### Toxicity

Investigators from each participating institution submitted toxicity data for the patients they entered in the study. When a flow sheet suggested serious toxicity, a physician from the study committee contacted the local investigator by phone and obtained more thorough information. When flow sheets made no mention of a problem, none was presumed, with the exception of asymptomatic hearing loss. Only patients with normal audiometry results were considered to have normal hearing.

## RESULTS

### Evaluable Patients

The study was started in June 1977 and was closed to patient entry in October 1980; 155 patients were

[3]Criteria for evaluating chemotherapy for acute leukemia in childhood. Adopted by CCSG, Sept 1963.

entered. Six patients were ineligible: five never received DDP, and one had no measurable tumor. Thirty-five patients were inevaluable: data were too early for evaluation for four; 13 had early death for reasons not apparently related to DDP toxicity; and 18 received only one infusion of DDP because of the preference of the patient or investigator (generally, the patients found effects of the drug to be intolerable or the physician chose not to persist in the face of progressive disease after one infusion).

A total of 114 patients were evaluable (table 1). Of these, four patients were evaluable for toxicity only, because three received only one infusion due to toxicity apparently related to DDP and one had no posttherapy evaluation of extent of disease.

Patients were considered to be evaluable for each toxicity item in table 2 if appropriate pre- and post-infusion studies were obtained and reported and if there was pre-existing evidence of that toxicity prior to the DDP infusion considered.

TABLE 1.—Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Age (yrs) | |
| 1 | 1 |
| 2 | 4 |
| 3 | 6 |
| 4 | 7 |
| 5 | 3 |
| 6 | 4 |
| 7 | 8 |
| 8 | 2 |
| 9 | 11 |
| 10 | 5 |
| 11 | 6 |
| 12 | 8 |
| 13 | 2 |
| 14 | 7 |
| 15 | 12 |
| 16 | 12 |
| 17 | 6 |
| 18 | 4 |
| 19 | 2 |
| 20 | 2 |
| 21 | 0 |
| 22 | 1 |
| 23 | 0 |
| 24 | 0 |
| 25 | 0 |
| 26 | 1 |
| 27 | 0 |
| 28 | 0 |
| No. of infusions | |
| 1 | 4 |
| 2 | 44 |
| 3 | 31 |
| 4 | 13 |
| 5 | 10 |
| 6 | 5 |
| 7 | 4 |
| 8 | 1 |
| 9 | 1 |
| 10 | 1 |

## Tumor Response

Three responses were seen among 18 patients with osteogenic sarcoma, one CR and two PRs (table 3); these are reported elsewhere (12).

There were one CR and two PRs among 16 patients with rhabdomyosarcoma. The CR occurred in a 4-year-old girl with embryonal rhabdomyosarcoma and a lesion at the base of the right lung, observed in a chest roentgenogram. After one infusion, the lesion appeared to have enlarged and the dose was increased. The lesion then disappeared. Despite continuation of DDP, the lesion reappeared after 2 months. A thoracotomy demonstrated it to be diaphragmatic rather than pulmonary. No other lesion was noted. The second patient was a 12-year-old boy who had pleomorphic rhabdomyosarcoma confined to the abdomen. His mass regressed well over 50%, as shown by palpation, after two infusions, but recurred after 2 months. The third patient was a 14-year-old boy with pelvic rhabdomyosarcoma with local recurrence and lung metastases. After two infusions of DDP, his chest roentgenogram showed a > 50% improvement. Abdominal girth decreased from 70 cm to 66 cm at the umbilicus. This response lasted 2 months, when therapy was interrupted because of renal toxicity. Among three additional patients with undifferentiated sarcoma, one patient had a PR; after one infusion, CT scan showed marked regression of a locally recurrent retro-orbital tumor. DDP was stopped after two infusions because of partial hearing loss, but the response lasted 4 months.

Three PRs were seen among 16 patients with Wilms' tumor. Two of the three patients had classic histology; after one infusion of DDP, chest roentgenograms in both patients demonstrated regression of lung metastases. Responses lasted 2 and 4 months. The third patient had sarcomatous histology, and after two courses, CT scan showed almost total resolution of a 2 × 2-cm paramediastinal mass; bone scan showed stabilization of bone metastases, and bone pain was eradicated. The PR lasted 5 months with therapy.

Two PRs were seen among 13 patients with Ewing's sarcoma. Chest roentgenogram showed that the patients, aged 22 and 27 years, achieved a > 50% regression of lung metastases that lasted 3 and 6 weeks, respectively.

PRs were seen in a patient with hepatocellular carcinoma and in one with hepatoblastoma. These were the only patients with liver tumors who received adequate trials of DDP. Both responses were

TABLE 2.—Toxicity

| Toxic effect | No. of evaluable patients | No. of patients with grade — 0 | 1+ | 2+ | 3+ | 4+ |
|---|---|---|---|---|---|---|
| Renal toxicity* | 99 | 73 | 7 | 10 | 4 | 5 |
| Hearing, symptomatic† | 114 | 106 | 2 | 4 | 1 | 1 |
| Anemia‡ | 36 | 9 | 18 | 9 | — | — |
| Thrombocytopenia§ | 91 | 48 | 19 | 22 | 1 | 1 |
| Neutropenia¶ | 94 | 70 | 10 | 9 | 2 | 3 |
| Hypocalcemia** | 61 | 35 | 14 | 11 | | 1 |
| Hypomagnesemia†† | 60 | 42 | 12 | 5 | | 1 |

*1+ = casts without other changes, or mild transient elevation of creatinine or BUN; 2+ = 30% decrease in creatinine clearance and no change in creatinine or BUN; 3+ = 30% decrease in creatinine clearance and mild elevation in creatinine (< 2.0 mg/100 ml) or BUN (< 40 mg/100 ml); 4+ = 30% decrease in creatinine clearance and severe elevation in creatinine (≥ 2.0 mg/100 ml) or BUN (≥ 40 mg/100 ml).

†1+ = tinnitus, no hearing loss; 2+ = transient partial hearing loss; 3+ = severe persistent hearing loss; 4+ = severe persistent hearing loss requiring hearing aid.

‡1+ = Hgb < 12 g/100 ml; 2+ = Hgb < 9 g/100 ml.

§1+ = platelet count < 150,000/$mm^3$; 2+ = platelet count < 100,000/$mm^3$; 3+ = platelet count < 50,000/$mm^3$; 4+ = platelet count < 25,000/$mm^3$.

¶1+ = absolute neutrophil count (ANC) < 1500/$mm^3$; 2+ = ANC < 1000/$mm^3$; 3+ = ANC < 500/$mm^3$; 4+ = ANC < 250/$mm^3$.

**1+ = $Ca^{2+}$ < 9 mg/100 ml, asymptomatic; 2+ = $Ca^{2+}$ < 8 mg/100 ml, asymptomatic; 3+ = $Ca^{2+}$ < 8 mg/100 ml, mild self-limited symptoms; 4+ = $Ca^{2+}$ < 8 mg/100 ml, severe symptoms.

††1+ = $Mg^{2+}$ < 1.5 mEq/liter, asymptomatic; 2+ = $Mg^{2+}$ < 1.2 mEq/liter, asymptomatic; 3+ = $Mg^{2+}$ < 1.2 mEq/liter, mild self-limited symptoms; 4+ = $Mg^{2+}$ < 1.2 mEq/liter, severe symptoms.

characterized by markedly decreased liver size on palpation, improvement in the liver-spleen scan, and decrease in alpha-fetoprotein. The PR in the patient with hepatocellular carcinoma lasted 6 months. The patient with hepatoblastoma had lung metastases when he entered the study. These patients did not have disease progression during 4 months of therapy. DDP appeared to have given both patients good clinical palliation and to have provided solid laboratory evidence of tumor regression.

Single PRs were obtained in neuroblastoma, ovarian teratoma, thymoma, and embryonal carcinoma of the mediastinum. In addition, 35 patients with 21 different tumors, including eight brain tumors, received DDP without response.

## Toxicity

Twenty-six of 99 patients (27%) developed some evidence of renal toxicity (table 2). Two patients had urinary casts, with no change in creatinine clearance. Five had a transient (< 3 weeks) mild elevation in BUN (< 40 mg/100 ml) or creatinine (< 2.0 mg/100 ml) and received further DDP without incident once the value had returned to normal. Nineteen patients had a persistent 30% decline in creatinine clearance; ten had no elevation of BUN or creatinine, four had a mild elevation, and five had a marked elevation of BUN (> 40 mg/100 ml) or creatinine (> 2.0 mg/100 ml). Two of the five had received further DDP despite persistent mild elevation of the serum creatinine following previous DDP administration. The highest creatinine level was 11.2 mg/100 ml. This decreased over 1 month to 1.1 mg/100 ml. No patient required dialysis. In general, renal status improved once DDP was stopped; however, extensive long-term followup data are not available.

Two patients had renal toxic effects after only one infusion of DDP. One had received 30% more DDP at a dose of 3 mg/kg than he would have received at a dose of 90 mg/$m^2$.

Of the remaining 97 patients who received at least two infusions of DDP, 11 of the 25 who received at least one infusion at a dose of 4.5 mg/kg had renal toxic effects, compared to the 13 of 72 who received no infusion > 3.0 mg/kg ($P < 0.5$). The median age of the 25 was 2 years older (13 vs 11) than that of the entire group, and the median number of infusions was one greater (four vs three) than that of the entire group. Three of 18 patients with Wilms' tumor and only one kidney had evidence of renal toxicity: one transient, one mild, and one marked.

The protocol recommended that each patient undergo baseline audiometry, but compliance was poor. The investigators submitted audiometry data for only 18 patients with no symptomatic hearing problems. Eight of 18 patients (44%) showed high-frequency hearing loss. Two additional patients had tinnitus that resolved despite further DDP. Six patients had symptomatic hearing loss. Of these, four had a transient partial loss and two had a severe

TABLE 3.—Trials and responses*

| Type of tumor | Total | To early for evaluation | Ineligible | Early death | Inadequate trial | Evaluable for toxicity only | Evaluable for response and toxicity | Response duration (mos) |
|---|---|---|---|---|---|---|---|---|
| Rhabdomyosarcoma | 24 | | | 3 | 5 | | 16 | 3(2†,2,2) |
| Wilms' tumor | 23 | | 2 | 2 | 1 | 2 | 16 | 3(4,2,5) |
| Osteogenic sarcoma | 19 | | 1 | | | | 18 | 3(7+,7,2†) |
| Ewing's sarcoma | 14 | | | | | 1 | 13 | 2(1,1) |
| Neuroblastoma | 9 | 1 | 1 | 1 | 2 | | 4 | 1(2) |
| Undifferentiated sarcoma | 6 | 1 | | 1 | 1 | | 3 | 1(4) |
| Non-Hodgkin's lymphoma | 5 | | | 1 | 1 | | 3 | |
| Hepatoblastoma | 4 | 1 | 1 | 1 | | | 1 | 1(4+) |
| Medulloblastoma | 4 | | | | 1 | | 3 | |
| Neurofibrosarcoma | 4 | | | | | | 4 | |
| Ovarian teratoma | 4 | | 1 | | 2 | | 1 | 1(3) |
| Retinoblastoma | 4 | | | | | | 4 | |
| Hodgkin's lymphoma | 3 | | | 1 | 1 | | 1 | |
| Synovial sarcoma | 3 | | | | | | 3 | |
| Adrenal carcinoma | 2 | | | | | | 2 | |
| Chondrosarcoma | 2 | | | | 1 | | 1 | |
| Giant cell tumor | 2 | | | 1 | | | 1 | |
| Hepatocellular carcinoma | 2 | | | | | 1 | 1 | 1(6) |
| Pineal tumor | 2 | | | | | | 2 | |
| Sacrococcygeal teratoma | 2 | | | | 1 | | 1 | |
| Acute nonlymphocytic leukemia | 1 | | | | 1 | | | |
| Astrocytoma | 1 | | | 1 | | | | |
| Carcinoma, not otherwise specified | 1 | | | | | | 1 | |
| Brain stem tumor | 1 | | | | | | 1 | |
| Brain dysgerminoma | 1 | | | | 1 | | | |
| Ependymoma | 1 | 1 | | | | | | |
| Extraosseous Ewing's sarcoma | 1 | | | | | | 1 | |
| Fibromyxosarcoma | 1 | | | | | | 1 | |
| Glioma | 1 | | | | | | 1 | |
| Lymphoepithelioma | 1 | | | | | | 1 | |
| Malignant fibrous histiocytoma | 1 | | | | | | 1 | |
| Mediastinal embryonal carcinoma | 1 | | | | | | 1 | 1(6) |
| Nasopharyngeal epidermoid carcinoma | 1 | | | 1 | | | | |
| Oligodendroglioma | 1 | | | | | | 1 | |
| Retroperitoneal teratoma | 1 | | | | | | 1 | |
| Small cell sarcoma | 1 | | | | | | 1 | |
| Thymoma | 1 | | | | | | 1 | 1(1) |
| Total | 155 | 4 | 6 | 13 | 18 | 4 | 110 | |

*Values = No. of patients.
†CRs; all other responses were PR.

persistent (> 3 weeks) loss; one of these required a hearing aid. One patient had a severe persistent hearing loss after one infusion of DDP. She had received 30% more drug at a dose of 3 mg/kg than she would have received at a dose of 90 mg/m².

Only patients with no bone marrow involvement by tumor and no preexisting anemia, neutropenia, or thrombocytopenia were evaluated for myelosuppression. Patients with active bleeding were not evaluated for anemia. Anemia was not evaluable for 78 of 114 patients.

Some degree of thrombocytopenia was common (43 of 91 patients), but only one patient had platelet counts < 25,000/mm³. Similarly, neutropenia was common (34 of 94 patients), but only 14 patients had ANCs < 1000/mm³; five of these were < 500/mm³. Myelosuppression increased in patients who received further DDP despite persistent neutropenia or thrombocytopenia following a previous infusion.

Levels of serum calcium and magnesium could be determined for 61 and 60 patients, respectively; ie, adequate data were supplied by the investigators and the patients were neither hypocalcemic ($Ca^{2+}$ < 9 mg/100 ml) nor hypomagnesemic ($Mg^{2+}$ < 1.5 mmol/liter) before treatment. After receiving DDP, 17 patients had hypocalcemia alone; nine had hypomagnesemia alone; and nine had both. Only one patient had symptoms: tetany and carpopedal spasm. She had a magnesium level of 0.5 mEq/liter and a calcium level of 6.0 mg/100 ml, which recovered with replacement therapy. Hypocalcemia and/or hypomagnesemia were most severe in patients with persistent hypocalcemia or hypomagnesemia following previous DDP.

Investigators reported nausea and vomiting with 66 of 373 infusions. Two patients had congestive heart failure. One had previously received 760 mg/m² of doxorubicin. One week after DDP and 2 months after the last dose of doxorubicin, he developed pulmonary edema and died 3 weeks later. The other had received 430 mg/m² of doxorubicin and pulmonary irradiation. He developed massive edema 3 weeks after his only dose of DDP and 6 weeks after his last dose of doxorubicin and died 3 weeks later of progressive disease.

Two patients had allergic reactions: one had total-body erythema 2 days after DDP; the other had erythema of the upper part of his body during an infusion. Symptoms were controlled with diphenhydramine. One of the patients completed the infusion without further problem.

## DISCUSSION

The CCSG data presented here and elsewhere corroborate those of Kamalakar et al (8), Ochs et al (9), and Nitschke et al (10,11), demonstrating DDP activity in osteogenic sarcoma and neuroblastoma, and those of Tekuzman et al (13), suggesting DDP activity in Wilms' tumor. Phase III investigations of DDP as a first- or second-line agent are underway. Ettinger et al (14) have reported encouraging early data on the combination of doxorubicin and DDP in newly diagnosed osteogenic sarcoma. CCSG is investigating the combination of DDP and VM-26 in recurrent neuroblastoma[4] and DDP and methotrexate in recurrent or metastatic osteogenic sarcoma.[5]

Three responses are reported here in rhabdomyosarcoma and two in Ewing's sarcoma. Nitschke et al (10,11) found no responders among six patients with rhabdomyosarcoma and ten patients with Ewing's sarcoma. Various doses were used: 15–20 mg/m²/day × 5 every 3–4 weeks; 1 mg/kg/week; 50 mg/m²/week; 60 mg/m²/day × 2 every 3–4 weeks; and 90 mg/m² every 3 weeks. Few patients received the last two doses, and perhaps in rhabdomyosarcoma and Ewing's sarcoma, a dose of 3.0 mg/kg or 90 mg/m² is necessary for response. However, among all the patients reported here only one of 25 patients who failed to respond at a dose of 3.0 mg/kg responded when the dose was increased to 4.5 mg/kg. The responses obtained were generally of short duration and may represent a minimal level of activity with no clinical significance. Further studies are required.

A single PR does not establish drug activity. In 1973, Talley et al (15) reported a PR in thymoma, and in 1975, Hill et al (16) reported a PR in an adult with hepatocellular carcinoma. The additional cases reported here add weight to these earlier reports. The responses in hepatocellular carcinoma and hepatoblastoma were particularly gratifying. Both were relatively durable and accompanied by significant palliation. In both the rabbit and the dog, platinum is concentrated in the liver after DDP infusion, reaching levels four times those found in skeletal muscle (17).

Mild toxicity was common. Severe toxicity was rare. Twenty-six of 99 patients had some degree of renal toxicity, but only five had creatinine levels > 2.0 mg/100 ml. Asymptomatic hearing loss was

[4]Baum E, Greenberg L, Ramsay N, et al. Children's Cancer Study Group, protocol CCG-372—evaluation of *cis* platinum diammine dichloride (CPDD) (NSC-119875) and 4-demethyl-epidophyllotoxin-β-D-thenglidene glucoside (VM-26) (NSC-112810) for the treatment of recurrent stage IV neuroblastoma of childhood, Jan 1978.

[5]Baum E, Morgan E, Bleyer A, et al. Children's Cancer Study Group, protocol CCG-781P—chemotherapy (methotrexate NSC-740, citrovorin factor NSC-3590, vincristine NSC-67574, and *cis* platinum diammine dichloride NSC-119875) for treatment of osteogenic sarcoma in children failing CCG-741 or other standard regimen, Mar 1978.

common (eight of 18 patients tested, 44%) but symptomatic hearing loss occurred in only six of 114 patients and it was severe and persistent in only two. Only five patients developed severe neutropenia (ANCs < 500/mm³) and only one developed severe thrombocytopenia (platelet count < 25,000/mm³). Myelosuppression was not life-threatening in any patient. Unlike the more troublesome experience reported by Pratt et al (18), only one patient was symptomatic from hypocalcemia or hypomagnesemia. The two instances of congestive heart failure might well be more closely related to anthracycline administration and cardiac irradiation. Neither of the allergic reactions was serious; both were easily controlled.

Two patients had significant renal toxic effects, and one had significant hearing loss after only one infusion of DDP with no apparent protocol violations. No associated aminoglycoside administration was reported. Calculating the dose both as 3 mg/kg and 90 mg/m² showed that two of three patients received 30% more DDP at a dose of 3 mg/kg than they would have received at a dose of 90 mg/m². The variation in the number of infusions administered and the relatively low incidence of toxicity prevent any statistically significant conclusions. However, when DDP was administered in a per-kilogram dose, older children received more and younger children received less than when the dose was calculated per meter squared. The amount of drug given varied ±40% when a dose of 3.0 mg/kg was compared with a dose of 90 mg/m². Otherwise, toxicity appears to be cumulative. When further DDP was administered despite evidence of persisting toxicity, toxic effects tended to increase. Others have found renal toxicity to be cumulative (13,14). Kinetic data (18) suggest that blood levels of DDP may still be at 5% peak levels 3 weeks after an infusion. Perhaps administration every 3 weeks is too frequent.

Among patients who received at least two infusions of DDP, 11 of the 25 who received at least one infusion at a dose of 4.5 mg/kg had renal toxic effects compared to 13 of the 72 who received DDP at a dose no greater than 3.0 mg/kg. However, patients who received DDP at a dose of 4.5 mg/kg received a median of four infusions of DDP, compared to three for those who did not.

Nausea and vomiting were reported less frequently than expected. All patients observed in Children's Memorial Hospital, Chicago, had nausea and most had some vomiting, but this complication seemed much the same as for a variety of other chemotherapeutic agents. Perhaps the other investigators did not consider it unusual enough to report.

Cooperative studies such as this one have advantages and disadvantages. Patient accrual is facilitated, but the study committee surrenders some surveillance. While the investigators complied with the protocol in general, compliance was less than desired with regard to audiometry and diligent completion of the flow sheets. Major toxic effects, as suggested by the notes on the flow sheets, were investigated by telephone contact, but where no notation existed, no toxicity could be presumed.

In conclusion, DDP administered at a dose of 3.0 mg/kg with aggressive hydration and diuresis every 3 weeks has activity, not only in neuroblastoma and osteogenic sarcoma, but also in Wilms' tumor, rhabdomyosarcoma, and Ewing's sarcoma. Phase III trials are needed to determine the role of DDP in the therapy of childhood cancer. Mild toxicity is common and includes elevation of BUN and/or creatinine, hearing loss, hypocalcemia and hypomagnesemia, and myelosuppression. Future trials should require more thorough audiometric surveillance. Severe toxicity was rare when DDP was administered according to protocol.

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# Phase II Study of Diglycoaldehyde in Malignant Melanomas and Soft Tissue Sarcomas [1,2]

**Gerald J. Vosika,* Kathleen Briscoe, Robert W. Carey, Joseph F. O'Donnell, Michael C. Perry, Daniel Budman, Frederick Richards, III, and Morton Coleman [2]**

## SUMMARY

**A phase II study of diglycoaldehyde was conducted in 42 patients with malignant melanoma and soft tissue sarcoma. All patients received diglycoaldehyde at a dose of 2 g/m² iv over 6 hours daily for 3 days. This was repeated every 28 days. Major toxic effects included phlebitis and azotemia, requiring dialysis in one patient. Of the 42 patients, 38 were evaluable for response. One objective response occurred in a patient with malignant melanoma. Diglycoaldehyde at this dose and schedule appears to have no role in the management of malignant melanoma or soft tissue sarcoma.**

[Cancer Treat Rep 65:823–825, 1981]

Inosine dialdehyde, commonly known as diglycoaldehyde, is the periodate oxidation product of the purine nucleoside, inosine. In diglycoaldehyde, carbons 2 and 3 of the sugar moiety are oxidized to formyl groups resulting in cleavage of the ring (1).

Diglycoaldehyde has demonstrated preclinical antitumor activity against L1210, P388, and AKR murine leukemias.[3] Minimal activity was also seen against the Lewis lung carcinoma. There was no activity against the Walker 256 carcinosarcoma, B16 melanoma, or rodent ependymoblastoma. The drug was most effective when given on a daily dose schedule.[3] The mechanism of action is primarily inhibition of RNA synthesis, with some inhibition of DNA and protein synthesis (2–6).

Three phase II studies of diglycoaldehyde have been performed in 24 children[4] and in 49 adults (7).[5] Doses ranged from 30 mg/m² to 2 g/m² daily for 5 days. In one study, a dose of 1.2 g/m² was given daily for 10 days. Side effects included nausea, vomiting, hypocalcemia, proteinuria, phlebitis, and the development of a positive Coombs' test. In these phase I studies, objective responses were noted in patients with acute lymphatic leukemia (three patients), acute myelogenous leukemia (one), lymphoma (one), and oat cell carcinoma (one). Based on these data a phase II study was initiated.

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[2]This study is from the New Agents Committee for the Cancer and Leukemia Group B and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: University of Minnesota Medical School, Minneapolis (*G. J. Vosika* and *K. Briscoe*, CA-16450); Massachusetts General Hospital, Boston (*R. W. Carey*, CA-12449); Dartmouth Medical School, Hanover, NH (*J. F. O'Donnell*, CA-04326); University of Missouri Medical School, Columbia (*M. C. Perry*, CA-12046); Northshore University Hospital-Cornell Medical Center, Manhasset, NY (*D. Budman*); New York Hospital-Cornell Medical Center, NY (*M. Coleman*, CA-07968); and Bowman-Gray School of Medicine, Winston-Salem, NC (*F. Richards, III*, CA-03927). Other participating institutions include Upstate Medical Center, Syracuse, NY (*A. Gottlieb*, CA-21060); University of California, San Diego (*M. Green*, CA-11789); and Jewish General Hospital, Montreal, Quebec, Canada (*R. Margolese*).

**Reprint requests to:* G. J. Vosika, MD, Department of Medicine, University of North Dakota, c/o Veterans Administration Hospital, Elm St N and 21st Ave, Fargo, ND 58102.

[3]Screening data from Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[4]Helman L, and Slavik M. Clinical brochure: diglycoaldehyde (NSC-118994). Prepared by the National Cancer Institute, Bethesda, MD, July 1976.

[5]Gottlieb J. Progress report of phase I meeting, Oct 3, 1974.

## MATERIALS AND METHODS

A total of 42 patients from nine participating Cancer and Leukemia Group B institutions were entered in the study. Four patients received less than one course of therapy and were ineligible for evaluation. There were 18 evaluable patients (11 males and seven females; median age, 54 years [range, 31–71]) with malignant melanoma. The median performance status (Karnofsky) was 90 (range, 50–100), and the mean was 86. Twelve of the 18 patients had received prior chemotherapy, which always included DTIC and sometimes included CCNU, vincristine, 5-FU, cycloserine, or vindesine. Eight patients had received prior immunotherapy, usually BCG or methanol-extracted residue. The mean time from diagnosis to disease recurrence in these patients was 10 months (range, 2–30). Six patients had cutaneous, subcutaneous, or nodal disease only, four had pulmonary disease, six had liver or abdominal disease, and two had bone disease.

There were 20 evaluable patients with sarcoma. This included patients with leiomyosarcoma (six patients), fibrosarcoma (five), synovial cell sarcoma (two), mesenchymoma (two), undefined sarcoma (two), and one each with rhabdomyosarcoma, liposarcoma, or spindle cell sarcoma. Of the 22 available patients, there were 12 males and ten females. The median age was 47 years (range, 32–79). The median performance status was 70 (range, 30–100). Fourteen of these patients had received palliative radiation therapy. Twenty-one of the 22 patients had received prior chemotherapy. All 21 patients had received doxorubicin. Seventeen patients had combinations of drugs, most frequently involving doxorubicin, vincristine, cyclophosphamide, and DTIC. A few patients (less than five) had received methotrexate, dactinomycin, cisplatin, or bleomycin. Eighteen of the 22 patients had pulmonary disease, with additional disease of the skin (four patients), nodes (three), liver (one), or bone (two). One patient had nodal disease only. Two patients had bone disease only and one had liver disease only. The median time from initial diagnosis to disease recurrence in this group was 12 months (range, 0–19).

All patients were routinely evaluated with physical examination, cbc, liver function tests, BUN, creatinine, creatinine clearance, Coombs' test, protime, partial thromboplastin time, thrombin time, and chest roentgenogram. Institutional approval of the protocol and informed patient consent were obtained prior to study.

Patients received diglycoaldehyde at a dose of 2 g/m$^2$ iv over 6 hours daily for 3 days. For use, the drug was dissolved in 1000 ml of 5% dextrose in water and buffered with sodium bicarbonate to a pH of 7.2.

### Toxicity

Seventy-five courses of diglycoaldehyde were administered. One patient received seven courses, two patients received three courses, 25 patients received two courses, and 12 patients received one course.

*Venous.*—The most common side effect was phlebitis, which occurred in 41% of the patients (table 1). In 11% of the patients this was severe and resulted in discontinuation of the drug in three patients. Phlebitis was largely prevented by the inclusion of heparin (5000 units) and hydrocortisone (100 mg) with each 1000-ml infusion.

*Hematologic.*—Hematologic toxicity was common (table 1). In 32% of the patients the hemoglobin decreased to < 12 g/dl. Leukopenia, with a wbc count of 2000–4000/mm$^3$, occurred in 26% of the patients. Thrombocytopenia, with a platelet count < 100,000/mm$^3$, was not seen. Eighteen of the patients had sequential Coombs' tests performed. In 11 of the 18 patients there was a conversion from negative to weakly positive direct Coombs' tests. There was no apparent relationship between conversion to positive and the occurrence of anemia. Seven of 11 patients with a positive Coombs' test had anemia. Four of 17 patients with a negative Coombs' test had anemia.

*Renal.*—An infrequent but serious complication was renal toxicity. Six patients experienced azotemia (creatinine > 1.2 mg/dl). In three (8%) of the patients there was severe renal toxicity, with creatinine > 4.0 mg/dl. One of these patients required

TABLE 1.—Toxicity of diglycoaldehyde in 40 patients

| Toxic effect | No. of patients (%) |
|---|---|
| Phlebitis | 17 (41) |
| Nausea and vomiting | 13 (32) |
| Renal creatinine (mg/dl) | |
| > 1.2 | 6 (16) |
| > 4.0 | 3 (8) |
| Hemoglobin (g/dl) | |
| < 12 | 12 (32) |
| < 9 | 3 (8) |
| Wbc count (cells/mm$^3$) | |
| < 4000 | 10 (26) |
| < 2000 | None |
| Platelet count (cells/mm$^3$) | |
| < 150,000 | 2 (5) |
| < 100,000 | None |

dialysis. In all patients the renal function abnormalities were ultimately reversible.

*Gastrointestinal.*—Nausea and/or vomiting occurred in 32% of the patients and was severe in 3%. Mild diarrhea occurred in 5% of the patients.

### Response

Of the 42 patients, four received less than a full course of therapy, with 38 patients evaluable for response. One objective response was seen in a patient with malignant melanoma with diffuse bone metastasis. Although there was no change after two courses, this patient had a gradual 90% improvement and continues to receive diglycoaldehyde after seven courses.

## DISCUSSION

The results of the current study showed one objective response among 18 patients with malignant melanoma and 27 with soft tissue sarcoma. From these data it was determined, using the binomial distribution, that the true response rate, with a 95% confidence level, for diglycoaldehyde in malignant melanoma is between 0.1% and 27%. Similarly, the true response rate in soft tissue sarcomas, if active, is $< 17\%$ (95% confidence).

In view of the difficulty of administration, toxicity, and a low probability of significant activity in patients with malignant melanoma and soft tissue sarcoma, the use of diglycoaldehyde in this dose and schedule for these diseases is not recommended.

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# Phase II Evaluation of PALA in Patients With Advanced Head and Neck Cancer[1,2]

E. T. Creagan,* W. C. Nichols, and J. R. O'Fallon[3]

## SUMMARY

**Nineteen patients with advanced head and neck cancer received 5.0 g/m² of PALA every 3 weeks. We achieved no objective regression in these patients, 17 of whom had had prior radiation therapy and five of whom had had no prior chemotherapy.**

[Cancer Treat Rep 65:827–829, 1981]

PALA is an inhibitor of aspartate transcarbamylase, a pivotal enzyme in de novo pyrimidine nucleotide biosynthesis (1). In experimental models, PALA has significant antitumor activity against a spectrum of advanced solid neoplasms (2). Early clinical trials identified that the dose-limiting toxic effects were rash and diarrhea (3,4); stomatitis was infrequent and myelosuppression was rare. Objective regressions have been achieved among diverse cancers including malignant melanoma and neurofibrosarcoma (5). Since an iv dose of 5.0 g/m² every 2 weeks produced substantial toxicity in approximately one third of the patients, we elected to use the same dose administered at 3-week intervals (T. Kubota, personal communication). This study assesses the role of PALA in patients with advanced head and neck cancer.

## MATERIALS AND METHODS

Nineteen patients with measurable or evaluable advanced head and neck cancer participated in the study (tables 1 and 2). All patients except two had received extensive radiation therapy (5000–6000 rads). Two patients had biopsy of the primary tumor followed by irradiation. All other patients had major surgical ablative procedures. Each patient was an acceptable candidate for cytotoxic treatment based on nutritional and hematologic status and was an ambulatory outpatient. Five patients had had no prior chemotherapy. The remaining 14 patients had received prior cytotoxic regimens with cisplatin as a single agent or in combination with cyclophosphamide and doxorubicin.

### Drug Administration

PALA was administered iv over 1 hour in 5% dextrose in water at a dose of 5.0 g/m² every 3 weeks. Patients were removed from the study at the first indication of disease progression.

### Evaluation

Prior to initial treatment and each subsequent course, patients had a complete general medical evaluation, routine hematologic and chemical parameters, a chest roentgenogram, and photographs of appropriate lesions.

For nine patients, an objective response was defined as a decrease of $\geq 50\%$ in the product of the longest perpendicular diameters of the most clearly measurable indicator lesion. There must have been no new areas of malignant disease or deterioration in weight, symptoms, or performance score. Progressive disease was defined as a $> 25\%$ increase in any indicator lesion. Objective stability was defined as a $\leq 25\%$ increase in any indicator lesion. The ten patients with evaluable but not clearly measurable disease were analyzed using previously reported criteria which show that a category of

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[3] Division of Medical Oncology (*E. T. Creagan* and *W. C. Nichols*) and Cancer Statistics Unit (*J. R. O'Fallon*), Mayo Clinic and Mayo Foundation, Rochester, MN.

**Reprint requests to:* E. T. Creagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, MN 55901.

TABLE 1.—Sites and histology of primary tumors

| Site | No. of patients |
|---|---|
| Oral cavity | |
| Squamous cell carcinoma | 7 |
| Adenoid cystic adenocarcinoma | 1 |
| Larynx | 3 |
| Cervical nodes (unknown primary) | 3 |
| Pharynx | |
| Squamous cell carcinoma | 1 |
| Lymphoepithelioma | 1 |
| Paranasal sinus | 2 |
| Parotid (squamous cell carcinoma) | 1 |

TABLE 2.—Patient characteristics

| Characteristic | No. of patients |
|---|---|
| Median age (yrs): 64 | |
| Sex (M:F) | 12:7 |
| ECOG performance score | |
| 0–1 | 8 |
| 2–3 | 11 |
| Prior treatment | |
| Radiation therapy (yes:no) | 17:2 |
| Chemotherapy (yes:no) | 14:5 |
| Indicator lesions | |
| Lymph nodes | 7 |
| Subcutaneous masses | 7 |
| Chest roentgenogram | 3 |
| Computerized axial tomography scan (sinus) | 2 |
| Grade | |
| 1–2 | 8 |
| 3–4 | 11 |

"assessible, nonmeasurable disease" provides valid and consistent response and survival data (6). The time to disease progression was calculated from the first day of treatment to date of progression or last followup.

## RESULTS

### Therapeutic

At the time of this analysis, all 19 patients had had documented disease progression, and 13 (68%) were dead.

No patient satisfied the criteria for an objective regression of either measurable or evaluable disease. Nine of the 19 patients (47%) showed immediate disease progression after only one course of PALA treatment. Seven patients received two courses, two had three courses, and one had five courses. Ten patients remained stable for a median duration of 50 days. Median time to disease progression was 43 days for all patients, while the estimated median survival was 15 weeks.

Covariate analyses using the Cox model (7) were performed to assess the associations of time to disease progression and time to death with five factors: age, sex, histologic grade (1, 2, 3, and 4), randomization performance status (0, 1, 2, and 3), and prior chemotherapy (yes vs no). Performance score was the only covariate significantly associated with both survival and time to disease progression ($P < 0.01$, $P < 0.025$, respectively). Patients with better performance scores had a substantial advantage compared to those with poorer performance scores (table 3).

### Toxicity

Approximately one third of the patients reported anorexia, which was related to the start of treatment in five of seven patients. Twenty percent reported diarrhea. Nausea and stomatitis occurred in two patients. Two other patients developed dermatologic complaints. One 57-year-old man had an intensely pruritic, erythematous, papular eruption over the scalp, which occurred 4 days after the first treatment. A 73-year-old female noticed a diffusely erythematous, pruritic, maculopapular rash on the trunk and thighs 1 week after receiving her fifth cycle of treatment. The toxicity in both patients resolved within 1 week without sequelae. One pa-

TABLE 3.—Performance score, time to disease progression, and survival

| Performance score | Time to disease progression | | Survival | |
|---|---|---|---|---|
| | < 6 wks | ≥ 6 wks | < 3 mos | ≥ 3 mos |
| 0–1 | 1 | 7 | 1 | 7 |
| 2–3 | 8 | 3 | 9 | 2 |
| Totals | 9 | 10 | 10 | 9 |

tient remarked about perioral paresthesias during the infusion, but this abated by prolonging the treatment to approximately 90 minutes. PALA had no obvious hematopoietic, hepatic, or renal toxicity.

## DISCUSSION

Despite adequate nutritional status and good performance scores, we were unable to demonstrate with our dose and schedule any therapeutic advantage from PALA for patients with advanced head and neck cancer. The probability is $< 5\%$ that the true response rate is $\geq 15\%$. Admittedly, most of our patients had had prior treatment, which usually portends a low response rate from subsequent treatment. Nevertheless, even among patients with favorable prognostic discriminants, there was no apparent benefit from PALA. On the other hand, our findings are clearly consistent with the observation of others that performance score is an important predictive factor of response to chemotherapy in advanced head and neck cancer (8).

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# Phase I Trial of Dihydroxyanthracenedione

David A. Van Echo,* Margaret Y. Whitacre, Joseph Aisner, and Peter H. Wiernik[2]

## SUMMARY

**Dihydroxyanthracenedione was given to 16 patients with solid tumors in a phase I clinical trial. The dose schedule used was a single daily dose for 3 consecutive days given every 3 weeks. The amount given ranged from 2 to 5 mg/m²/day. The dose-limiting toxic effect was moderate to severe leukopenia which occurred at a dose ≥ 4 mg/m²/day × 3. Thrombocytopenia was infrequent and did not require transfusion. Nonhematologic side effects were insignificant and included nausea, vomiting, and green-tinged urine. A minor tumor response was noted in a patient with fibrosarcoma. The recommended doses for solid tumor phase II studies are 4 mg/m²/day × 3 for good-risk patients and 3 mg/m²/day × 3 for poor-risk patients, given every 3 weeks.**

[Cancer Treat Rep 65:831–834, 1981]

Biologically active anthracycline antibiotics have been known and studied since the early 1950s when Brockmann et al (1) first discovered the red-pigmented rhodomycins. The orange-red anthracyclines daunorubicin and doxorubicin (ADM) have been the most extensively investigated because of their potent clinical antitumor activity (2) and were discovered by Di Marco et al (3) and Arcamone et al (4), respectively. The clinical effectiveness of daunorubicin and ADM has been limited, however, by dose-limiting side effects, particularly stomatitis, liver dysfunction, bone marrow suppression, and cumulative dose-dependent cardiac toxicity (5–7). To overcome the undesirable effects, structural modifications have been made to known anthracyclines, and new anthracyclines have been sought from the fermentation products of various *Streptomyces* species. Hopefully, these will be clinically useful and less toxic than previous anthracyclines.

Dihydroxyanthracenedione (DHAD) is one of a series of anthraquinone compounds currently being evaluated for antitumor activity. It has demonstrated antitumor activity in experimental mouse tumor systems. Although its mechanism of action has not been demonstrated, preliminary evidence suggests that DHAD is a DNA-reactive agent (8). In other studies, DHAD inhibited RNA and DNA synthesis in mouse lymphoma L51787 cells. When compared on an equimolar basis with ADM, DHAD is four to seven times more potent in inhibiting the incorporation of tritiated thymidine and uridine into these lymphoma tissue culture cells.[3] Studies in normal mouse bone marrow cells indicate that DHAD is non-cell-cycle specific in producing cytotoxicity in a manner similar to cyclophosphamide or ADM.[3]

Preclinical pharmacologic studies have only been performed in the dog and are incomplete. DHAD disappears rapidly from the plasma and < 1% appears in the urine during the 24 hours after a single dose.[3] Further studies in animals and man are currently in progress.

Preclinical antitumor studies indicate that DHAD is one of the most active compounds ever tested.[3] It has significant activity (increase in lifespan > 200%), with a high percent of "cures" (60–90-day survivors) in the following murine tumor systems:

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[2]Baltimore Cancer Research Program, Division of Cancer Treatment, National Cancer Institute, MD.

**Reprint requests to:* David A. Van Echo, MD, Baltimore Cancer Research Program, Division of Cancer Treatment, National Cancer Institute, University of Maryland Hospital, 22 S Greene St, Baltimore, MD 21201.

[3]Clinical brochure: anthracenedione (CL 232,315; NSC 301739). Prepared by the National Cancer Institute, Bethesda, MD, Mar 1979.

P388 and L1210 lymphoid leukemias; B16 melanoma, and colon tumor 26. DHAD also has activity in the ADM-resistant P388 leukemia, indicating a lack of cross resistance with ADM and little activity in the murine Lewis lung carcinoma and Ridgway osteogenic sarcoma.

Toxicology studies in beagles and monkeys indicate that DHAD exerts its main toxic effects on rapidly proliferating tissues (eg, bone marrow and mucous membranes), which is manifested by vomiting, diarrhea, anorexia, myelosuppression, and hemorrhage.[3]

The toxic dose low for dogs on a single-dose daily × 5 schedule is 0.52 mg/m², which normally would indicate a starting dose in man of 0.13 mg/m²/day × 3–5. However, Von Hoff et al (9) have already completed a phase I clinical trial of DHAD given iv on a once-every-4-week schedule. The maximal tolerated dose in that study was 12 mg/m². Toxic effects observed were nausea, vomiting, myelosuppression, and green-colored urine. The dose-related and dose-limiting toxic effect was myelosuppression. Wbc and platelet count nadirs occurred on Day 9 from treatment, with recovery by Day 21.

With this preliminary clinical evaluation available, a starting dose of 2 mg/m²/day × 3 is reasonable.

## MATERIALS AND METHODS

### Patient Selection and Evaluation

A total of 16 patients (12 males and four females), with a median age of 54 years (range, 32–67) and a median performance status of 70% (range, 40%–90%), received one or more courses of DHAD (total, 31 courses). All patients had solid tumors (table 1). Criteria for entry in the study required histologic proof of malignant disease, absence of conventional therapy, no chemotherapy or radiotherapy for at least 4 weeks prior to entry in the study (8 weeks in the case of nitrosoureas), and a minimum life expectancy of 6 weeks. Patients were expected to have acceptable blood cell counts (wbcs ≥ 4000/μl; platelets ≥ 100,000/μl) and normal hepatic and renal functions. Measurable disease was not required. Written informed consent was obtained from each patient. All patients had received extensive prior chemotherapy, including three who had received nitrosoureas or mitomycin. Three patients had received both radiotherapy and chemotherapy.

Baseline studies obtained before treatment included: hematocrit, wbc count with differential, platelet count, urinalysis, serum electrolytes, glucose, BUN, calcium, phosphorus, uric acid, creatinine, bilirubin, albumin, total protein, alkaline phosphatase, SGOT, LDH, prothrombin, partial thromboplastin time, and fibrinogen. During therapy, wbc and platelet counts were repeated twice weekly, and the coagulation and biochemical tests were repeated weekly.

### Drug and Schedule of Administration

DHAD was supplied by the Investigational Drug Branch, National Cancer Institute (Bethesda, MD), as a formulation in 5- or 10-ml ampules containing 0.5 mg/ml of the free base of DHAD. For use, the desired dose is added to 50 or 100 ml of 5% dextrose in water. Since the supplied solutions do not contain antibacterial preservatives, the DHAD was given within 4 hours after preparation. In this study, each dose of drug was administered iv over 10 minutes.

Patients received DHAD as inpatients or outpatients, depending on their overall medical condition. Pulse, temperature, and blood pressure were recorded prior to administration and at 15–30-minute invervals thereafter as indicated for 2–4 hours.

One course of treatment consisted of a single dose of DHAD given daily for 3 consecutive days. Courses were repeated every 3 weeks (table 2). At least three patients were treated at each nontoxic dose level and were observed for 2 weeks before being treated at a higher escalation dose. Five or six patients were treated at any dose level showing reversible toxicity. Two of the 16 patients who had

TABLE 1.—Tumor types in patients treated with DHAD

| Disease | No. of patients | No. of patients evaluable for response* |
|---|---|---|
| Soft tissue sarcoma | | |
| Fibrosarcoma | 2 | 2 |
| Leiomyosarcoma | 2 | 2 |
| Hemangeopericytoma | 1 | 1 |
| Malignant fibrous histiocytoma | 1 | |
| Mesothelioma, peritoneal | 1 | |
| Lung | | |
| Squamous cell carcinoma | 2 | 1 |
| Small cell carcinoma | 3 | 1 |
| Breast carcinoma | 1 | 1 |
| Stomach carcinoma | 1 | 1 |
| Colon carcinoma | 1 | |
| Transitional cell carcinoma, renal pelvis | 1 | 1 |
| Totals | 16 | 10 |

*Criteria for evaluation for response included measurable disease and at least 2 courses of DHAD.

TABLE 2.—Dose increments of DHAD and No. of patients treated at each level

| Dose ($mg/m^2$/day × 3) | Total No. of patients | No. of patients who received— 1 course | 2 courses | 3 courses |
|---|---|---|---|---|
| 2 | 4 | 4 | 3 | 0 |
| 3 | 3 | 3 | 3 | 2 |
| 4 | 6 | 6 | 3 | 0 |
| 5 | 5 | 5 | 2 | 0 |

received two or more courses of DHAD at a single dose level without undue toxicity and who had tumor stabilization and/or subjective improvement were escalated to the next available dose level. These two patients received one additional course at the new dose level.

## Toxicity

All 16 patients were evaluable for toxicity. Hematologic side effects are shown in table 3. Myelosuppression was the dose-related and dose-limiting toxic effect, which occurred in 11 patients (69%). The toxic effect on the wbc count was more pronounced than on the platelet count. Leukopenia (wbcs < 4000/μl) was almost absent at the 2-$mg/m^2$/day dose level and increased from moderate to severe with the 3–5-$mg/m^2$/day dose levels. At a dose of 3 $mg/m^2$, three of three patients developed a modest leukopenia, with return to pretreatment levels by Day 21. At a dose of 4 $mg/m^2$ the leukopenia was more pronounced (median, 1700 cells/μl; range, 900–3400), but recovery was still within 21 days. However, at the 5-$mg/m^2$ dose level the nadir wbc count was not only more severe (median, 700/μl; range, 500–1000), but recovery to ≥ 4000/μl required 30 days. The leukopenia observed was not cumulative. Insufficient numbers of patients had received prior radiotherapy to determine if this modality of treatment predisposed patients to myelosuppression with subsequent DHAD exposure. Thrombocytopenia occurred in three patients, each at a different dose level and always with leukopenia, and is probably related to the extent of prior myelosuppressive therapy (two of the three patients had received prior mitomycin).

Nonhematologic toxicity was very mild and included infrequent nausea (five patients), vomiting (three), and green-tinged urine (six). There was no clinical evidence of any hepatic, renal, cardiovascular, or allergic toxic effects in this study. Alopecia was not seen. DHAD is a known vesicant, but no instances of extravasation or local irritation were observed.

## Therapeutic Response

An objective tumor response (a < 50% reduction in the product of measurable perpendicular diameters) was seen in a patient with widely disseminated fibrosarcoma of the scalp. The patient had regression of several large subcutaneous nodules for 4 weeks when new metastatic lesions developed.

TABLE 3.—Hematologic toxicity

| Dose ($mg/m^2$/day × 3) | No. of patients | No. of evaluable courses | Wbc toxicity: Median nadir (cells × $10^3$/μl) | Wbc toxicity: Median days to nadir | Wbc toxicity: Median days to recovery | Platelet toxicity: Nadirs (< 150 × $10^3$ cells/μl) |
|---|---|---|---|---|---|---|
| 2 | 4 | 7 | 4.9 (1.2–7.9)* | 12 (10–14) | 21 | 69,107† |
| 3 | 3 | 6 | 3.6 (2.7–4.0) | 11 (8–15) | 21 (18–23) | |
| 4 | 6 | 8 | 1.7 (0.9–3.4) | 11 (7–15) | 21 (16–23) | 102,145 |
| 5 | 5 | 7 | 0.7 (0.5–1.0) | 12 (9–13) | 30 (16–30) | 13 |

*Nos. in parentheses = range.
†There was 1 patient at each level of thrombocytopenia.

## DISCUSSION

The results of this study indicate that DHAD has tolerable and reversible toxicity and can be given safely at a single daily dose of 4 mg/m² for 3 consecutive days every 3 weeks. At this dose level, the dose-limiting toxicity has been myelosuppression, primarily manifested as leukopenia. This usually occurred around Day 11. Complete recovery was noted by Days 16–23 (median, 21). There were no septic or bleeding episodes secondary to myelosuppression. The maximum number of courses given in our study was five. There was no evidence of cumulative toxicity. Patients with previous mitomycin treatment appeared to be more susceptible to myelosuppression, especially thrombocytopenia.

Other side effects were minimal and included nausea, vomiting, and green-tinged urine.

This drug is easily administered and well-tolerated. Further phase II studies are indicated. From these data, the recommended starting dose in good-risk patients with a high performance status and little or no prior myelosuppressive therapy is 4 mg/m²/day and 3 mg/m²/day for all others, given as a single-dose iv 10-minute infusion daily for 3 consecutive days, repeated every 21 days. Since DHAD has a dramatic effect on the wbc count, it might be a useful agent to test in the treatment of hematologic malignancies. Plans to incorporate this agent in phase II studies in acute leukemia and soft tissue sarcoma are currently being contemplated at the Baltimore Cancer Research Program.

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# Phase I Trial of PCNU Administered by 5-Day Courses[1,2]

**Robert H. Earhart,* Jim M. Koeller, and Hugh L. Davis[3,4]**

## SUMMARY

**PCNU was selected for clinical trials based on high activity in both standard and intracisternally transplanted murine tumors. PCNU was administered to 24 patients with refractory advanced solid tumors by courses of five daily iv injections every 6 weeks. The total dose ranged from 25 to 125 mg/m²/course. The major dose-limiting toxicity was reversible thrombocytopenia, with the nadir at 28–49 days and recovery by 2 weeks later. At a dose of 125 mg/m²/course, the mean nadir platelet count was 77 × $10^3$/mm³ (range, 16–201 × $10^3$/mm³). Recovery time was prolonged with successive courses in four patients, suggesting cumulative toxicity. The mean nadir of leukopenia at this dose was 2.6 × $10^3$ cells/mm³ (range, 1.2–5.0 × $10^3$ cells/mm³) and tended to occur with a later median at Day 44. Nausea and vomiting were unusually mild for a nitrosourea. Sporadic transaminasemia and elevated LDH may have been related to the vehicle, *N,N'*-dimethylacetamide. Other major organ toxic effects were not encountered, and there were no objective responses. PCNU was found to be a base-substitution mutagen in the *Salmonella typhimurium* assay. A starting dose of 125 mg/m², divided into five daily doses, is suggested for phase II trials in patients with no significant hematologic compromise from prior chemotherapy or radiation, and a dose of 75 mg/m² is recommended for all others.**

[Cancer Treat Rep 65:835–840, 1981]

The nitrosoureas form a family of drugs with qualitatively similar but quantitatively different antineoplastic effects and toxicity. Based on improved preclinical activity over BCNU in L1210 leukemia and Lewis lung carcinoma, as well as on oral administration, CCNU was evaluated clinically and eventually released. CCNU has superior clinical activity to that of BCNU in human lung tumors, and equivalent activity in brain tumors, melanomas, lymphomas, and breast cancer (2). Methyl-CCNU has been introduced based on improved animal solid tumor activity over both BCNU and CCNU, as well as increased lipid solubility. It has superior activity in human colon cancer, inferior activity in brain tumors and lymphomas, and probably equivalent activity against other human cancers (3). Streptozocin provided evidence that structural modifications in the nitrosourea series could greatly alter toxicity and led to the clinical trials of chlorozotocin, an analog with low carbamoylating activity and reduced nephrotoxicity. Initial phase II trials of chlorozotocin have failed to demonstrate a major improvement in therapeutic index over the other nitrosoureas, although direct comparison studies are needed.

PCNU is the most recent member of the nitrosourea group to enter into clinical trials. Sophisticated studies at the Southern Research Institute (Birmingham, AL) identified four important factors in prediction of nitrosourea activity against L1210

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[2]Supported by contract N01-CM-87208 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Earhart is the recipient of Junior Faculty Clinical Fellowship No. 388 from the American Cancer Society.

[3]Department of Human Oncology, Wisconsin Clinical Cancer Center, Madison.

[4]We thank Dr. E. G. Kendrick for performing Ames testing on PCNU.

**Reprint requests to:* Robert H. Earhart, MD, PhD, K4/532 Clinical Sciences Center, 600 Highland Ave, Madison, WI 53792.

leukemia: low chemical stability, low carbamoylating activity, high alkylating activity, and relatively high lipid solubility (4). Similar studies using the Lewis lung solid tumor established that PCNU had ideal lipophilicity (measured by octanol:water partition coefficient) for maximal activity in this system (5). PCNU has the lowest carbamoylating activity and highest alkylating activity of the clinically available nitrosoureas.[5] The confirmed activity of PCNU against the Lewis lung tumor (6) led to more extensive trials, in which PCNU proved to be the most active nitrosourea tested in a variety of animal neoplasms.[5] PCNU has significant activity against the intracisternally implanted murine ependymoblastoma; this activity often correlates with effectiveness against human melanoma and indicates potential effectiveness in brain tumors. Activity of PCNU was the best among a series of nitrosoureas (CCNU, streptozocin, methyl-CCNU, chlorozotocin, and BCNU) against intracisternal B16 melanoma, colon tumor 26, glioma 26, glioma 261, ependymoblastoma, L1210 leukemia, and P388 leukemia. It is also active in the standard B16 melanoma, colon tumor 26, L1210 leukemia, P388 leukemia, and Lewis lung tumor systems. Thus, there is excellent rationale for predicting that PCNU will provide further improvement in nitrosourea response rates in human cancer.

Toxicology studies in animals reported that dogs and monkeys displayed emesis, diarrhea, hyperthermia, and weight loss. Leukopenia, anemia, and reticulocytopenia were also observed, and dogs developed reversible thrombocytopenia at high doses. Elevated bromsulphalein retention, alkaline phosphatase, SGOT, and SGPT in both species were not clearly dose-related and may have been due to the vehicle (dimethylacetamide, 0.66%–1.0%, and propylene glycol, 6.6%–10%, in normal saline). Guinea pigs showed some local tissue injury with multiple injections, but single injections in guinea pigs and both single and multiple injections in rabbits produced no local damage. The mutagenic and carcinogenic activity of PCNU has not been assessed.

BCNU, CCNU, and methyl-CCNU are usually administered in single doses every 6 weeks, due to the delayed neutropenia nadir at 5–7 weeks. PCNU has completed phase I trials with this schedule (7), by daily × 2 administration every 6 weeks (8), and also on Days 1–3 every 6 weeks (10). However, CCNU has also been found to be effective and less toxic on an every-3-week schedule by the European Organization for Research on Treatment of Cancer (10). Chlorozotocin has produced responses with acceptable toxicity in phase I trials on a daily × 5 schedule every 6 weeks (11,12). In animal studies, PCNU was equally effective against the intracisternally inoculated murine ependymoblastoma as a single dose or on a daily × 5 schedule.[5] A trial of a daily × 5 schedule for PCNU in the clinical setting was consequently of interest and was undertaken by us (1) and by Memorial Sloan Kettering Cancer Center (New York, NY) (13) in two independent studies.

[5]Clinical brochure: PCNU. Prepared by the National Cancer Institute, Bethesda, MD, July 1978.

## MATERIALS AND METHODS

Patients with histologic evidence of advanced malignancy, who were not candidates for known effective regimens or protocol treatments and had recovered from toxic effects of prior treatment, were eligible for this study. Each patient had an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 3 and a life expectancy of at least 12 weeks. If patients had received previous chemotherapy, immunotherapy, and/or radiotherapy, a minimum of 3 weeks had to have elapsed since the last treatment. Patients were required to have adequate bone marrow function (wbc count ≥ 4000/mm$^3$, absolute granulocyte count ≥ 2000/mm$^3$, and platelet count ≥ 100,000/mm$^3$), adequate liver function (bilirubin < 2 mg/100 ml and prothrombin activity > 80% of normal), and adequate renal function (BUN ≤ 30 mg/100 ml, creatinine ≤ 1.5 mg/100 ml, and a creatinine clearance > 60 ml/minute). Informed consent indicating the investigational nature of the study was obtained from all patients according to institutional policy. Responses and toxicity were defined by standard ECOG criteria (14). Prior nitrosourea therapy did not disqualify patients from this study.

Pretreatment evaluation included a complete history and physical examination with documentation of metastatic disease and objective tumor measurement when possible. Laboratory studies included a cbc with differential, reticulocyte and platelet counts, chemistry panel (BUN, creatinine, SGOT, alkaline phosphatase, total protein, albumin, total bilirubin, LDH, calcium, phosphorus, and uric acid), creatinine clearance, urinalysis, electrocardiogram, and chest roentgenogram. Weekly cbcs and chemistry panels were obtained. Repeat physical examinations with tumor measurements were performed at 6-week intervals.

PCNU was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) in sterile 10-ml amber vials containing 50 mg of lyophilized powder. Each vial was reconstituted by addition of 0.25 ml of sterile *N*,*N*′-dimethylacetamide and agitation until the drug was com-

pletely dissolved. This solution was then diluted by addition of 2.25 ml of sterile propylene glycol to yield a solution containing 20 mg/ml of PCNU. The appropriate dose of this solution was added with aseptic technique to a volume of 5% dextrose injection, USP, so that the final PCNU concentration was ≤ 0.5 mg/ml. This aqueous solution was infused over 30–60 minutes into a freely flowing iv line. Treatments were repeated daily for 5 days every 6 weeks, provided that recovery from all toxicity due to prior treatments had ensued.

Based on a toxic dose low of 15 mg/m²/day for 5-day iv courses of PCNU in beagle dogs (*see* footnote 5), a starting dose of 5 mg/m²/day was selected. Escalation proceeded by a standard modified Fibonacci scheme, using doses of 5, 10, 15, and 25 mg/m²/day. Dose escalation within patients was prohibited, but doses were reduced to 75% or 50% of the initial level if myelosuppression was significant in the previous course. At least three patients were entered at each dose level and were observed for one full course before entry to the next level was permitted. If significant toxic effects were observed, at least three additional patients were entered at that level before escalation.

Specimens of PCNU as supplied by the National Cancer Institute were subjected to screening for mutagenicity with and without S-9 microsomal activation in *Salmonella typhimurium* strains TA-98 and TA-100, using 2-aminofluorene as a positive control and following standard published methods (15,16).

## RESULTS

Twenty-four patients were entered in this phase I trial. A total of 38 courses of PCNU were administered, and 36 of those courses were evaluable. One inevaluable patient was treated with one course of PCNU at 25 mg/m²/day × 5 days but was removed from the study on Week 4 by her personal physician and started on combination chemotherapy with methotrexate, vincristine, bleomycin, and prednisone for rapidly progressive nodular mixed non-Hodgkin's lymphoma. The second inevaluable patient received one course of PCNU but had a disease-related decline in performance status requiring hospitalization at another institution, where laboratory studies were inadequate to assess drug-related effects prior to his death. Patient characteristics are shown in table 1. Only one patient with no prior chemotherapy or radiation therapy was included. All other patients had received prior chemotherapy, usually with more than one agent, and nine had also received radiation therapy.

TABLE 1.—Patient characteristics

| Characteristics | | No. of patients |
|---|---|---|
| Total entered | | 24 |
| Total evaluable | | 22 |
| No. of courses completed: | 39 | |
| No. of courses evaluable: | 37 | |
| Tumor type | | |
| Lung | | 4 |
| Renal | | 6 |
| Colon | | 8 |
| Sarcoma | | 1 |
| Glioblastoma | | 1 |
| Mesothelioma | | 2 |
| Lymphoma | | 1 |
| Malignant melanoma | | 1 |
| Prior therapy | | |
| Chemotherapy only | | 14 |
| Radiation therapy only | | 0 |
| Both | | 9 |
| Neither | | 1 |
| Performance status (ECOG scale) | | |
| 0–1 | | 19 |
| 2–3 | | 5 |

## Toxicity

Myelosuppression proved to be the dose-limiting toxic effect in this phase I trial. As outlined in table 2, thrombocytopenia occurred more frequently and at lower doses than did leukopenia. Two of six courses at 25 mg/m²/course produced platelet count nadirs $< 100 \times 10^3$/mm³, but wbc count nadirs were 4.4 and 5.1 $\times 10^3$/mm³ in these courses. Indeed, no patient at this dose level or at 50 mg/m²/course had significant leukopenia. At 75 mg/m²/course, four of nine courses caused thrombocytopenia and one course produced leukopenia. Of 15 courses at a dose of 125 mg/m²/course, 12 were associated with thrombocytopenia and nine elicited leukopenia. Platelet count nadirs tended to occur at Weeks 4–5, while wbc count nadirs occurred at Weeks 5–7.

Nine patients received more than one course of PCNU; four of these patients began at the highest dose level. The hematologic data from these four patients are presented in figure 1. Patients A, C, and D required dose reductions, which did not materially alter the magnitude of their subsequent hematologic nadirs. Patients A, C, and D also showed prolongation of the time to recovery in successive courses, necessitating delays in treatment. Patient B exhibited increased thrombocytopenia following the fourth course at full dose and projected schedule. These data support the results of other investigators (7,8), suggesting cumulative hematologic toxicity of PCNU. Of the 13 patients who received

TABLE 2.—Hematologic toxicity

| Total dose (mg/m²/course) | No. of evaluable patients | No. of evaluable courses | Median wbc count nadir, cells × $10^3$/mm³ (range) | Median day of wbc nadir | Median platelet count nadir, cells × $10^3$/mm³ (range) | Median day of platelet nadir |
|---|---|---|---|---|---|---|
| 25 | 4 | 6 | 5.7 (4.4–7.4) | 30 | 252 (24–478) | 27 |
| 50 | 4 | 7 | 5.5 (3.7–8.2) | 46 | 230 (162–353) | 27 |
| 75 | 7 | 9 | 4.2 (3.1–4.6) | 38 | 105 (20–188) | 38 |
| 125 | 7 | 15 | 2.6 (1.2–5.0) | 44 | 71 (11–201) | 32 |

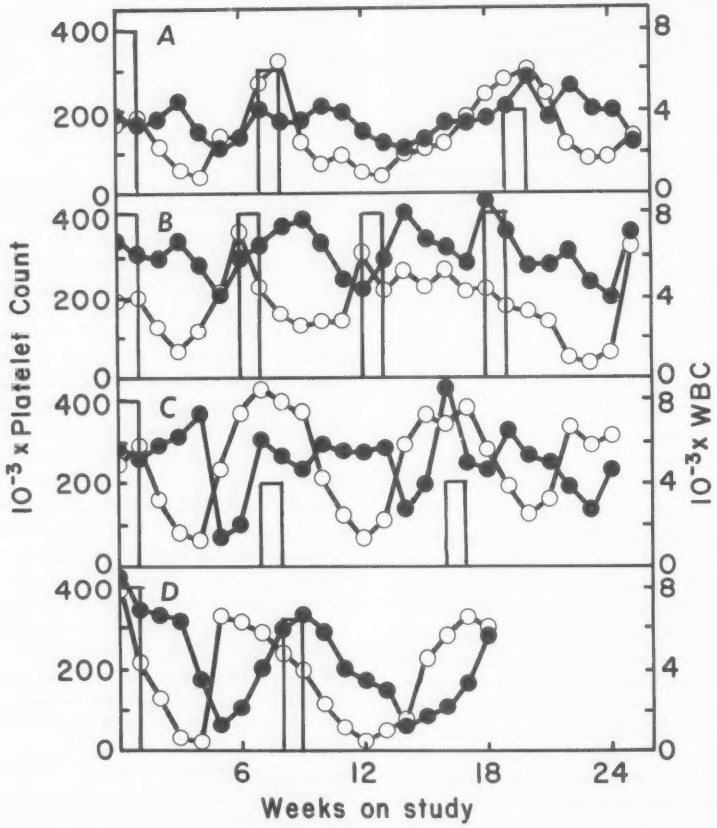


FIGURE 1.—Hematologic toxicity and recovery in 4 patients (A, B, C, and D) receiving PCNU at a starting dose of 125 mg/m²/course. Bars = relative dose of drug and time of administration. Weekly platelet count (○) and wbc count (●) data are shown.

TABLE 3.—Nonhematologic toxic effects of PCNU (incidence/evaluable cases)

| Total dose (mg/m²/course) | No. of evaluable patients | No. of patients with— | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Nausea and vomiting | Diarrhea | Stomatitis | Hepatic toxicity* | Renal toxicity | Anorexia | Malaise |
| 25 | 4 | 2 | 3 | 0 | 1 | 0 | 2 | 2 |
| 50 | 4 | 2 | 0 | 0 | 1 | 0 | 3 | 3 |
| 75 | 7 | 4 | 0 | 0 | 2 | 0 | 5 | 5 |
| 125 | 7 | 2 | 0 | 0 | 0 | 0 | 2 | 2 |

*Elevated SGOT and LDH.

only one course, five were withdrawn due to rapidly progressive disease and eight had disease-related decreases in performance status, which rendered them inappropriate candidates for further treatment of any kind.

Acute nonhematologic toxic effects of PCNU at this schedule were milder than expected for a nitrosourea. Table 3 summarizes the incidence of observed and anticipated types of acute toxicity. Nausea and emesis were mild and easily controlled with prochlorperazine. Elevations of SGOT and LDH were transient, not dose-related, and possibly associated with the vehicle. Anorexia and malaise were more common and only somewhat dose-related, as shown. No extravasations occurred, and no phlebitis was noted.

### Responses

No objective responses meeting standard ECOG criteria were observed in this phase I trial. One patient with renal cell carcinoma exhibited temporary improvement in disease-related hypercalcemia.

### Mutagenic Activity

PCNU was inactive against the frameshift mutant *Salmonella typhimurium* strain TA-98. The drug produced 0.89 revertants/nmol/$10^8$ cells of the TA-100 strain, a base-substitution mutant. This represents 14% of the observed activity of the positive control 2-aminofluorene on a molar basis.

## DISCUSSION

Preliminary results of phase I trials of PCNU have shown remarkable homogeneity in a variety of schedules. As a single dose every 6–8 weeks, thrombocytopenia was dose-limiting at 110 mg/m² for patients with adequate marrow reserve and at 60 mg/m² for patients with compromised marrow (7). Significant granulocytopenia was also noted, as were nausea, emesis, malaise, mild transaminasemia, and diarrhea, as in the present study. Variant findings included mild hypotension, stomatitis, and skin rash, as well as the observation of objective responses in astrocytoma, medulloblastoma, melanoma, colon carcinoma, and Hodgkin's disease. Marrow toxicity was thought to be cumulative. A maximum tolerated total dose of 80–100 mg/m² was reported on a daily × 2 schedule, with dose-limiting granulocytopenia that appeared to be cumulative and more severe in patients who had had prior chemotherapy (8). Renal and hepatic toxicity was not seen, and nausea and vomiting was characterized as mild. Single responses were seen in glioblastoma and colon carcinoma. On a daily × 3 schedule, a maximum tolerated total dose of 90–100 mg/m² was recommended, with reduction to 75 mg/m² in patients with compromised marrows (9). Again, myelosuppression was dose-limiting and was more severe in patients with prior chemotherapy. Renal and hepatic injury was not found, and nausea and vomiting was mild. A single partial response was seen in a patient with hypernephroma. In a trial similar to ours, a maximum tolerated total dose of 100–125 mg/m² was found on a 5-day schedule (13). Thrombocytopenia was more significant than leukopenia, and nausea was mild. No responses were noted.

Our results are entirely consistent with these preliminary reports and suggest a maximum tolerated dose of 25 mg/m²/day on a 5-day schedule in patients with good marrow function. Under these conditions, a syndrome of anorexia and malaise will be observed in many patients, and sporadic elevations of SGOT and LDH may be due to the vehicle. Pulmonary and/or renal toxic effects similar to those of the other nitrosoureas may be seen with more widespread use of PCNU in future trials. It should be emphasized that nausea and vomiting were unusually mild in all reports, to date.

Most of our patients who had received prior therapy with nitrosoureas, mitomycin, mitolactol, or other agents that induce marrow compromise were clus-

tered at the lower dose levels. Although we were unable to associate increased hematologic toxicity from PCNU with marrow compromise on a statistical basis, it is probably prudent to start such patients at a reduced initial dose of 75 mg/m$^2$/course and escalate in the next course only if this dose is well-tolerated. Phase II protocols should also contain de-escalation rules.

It bears re-emphasis that the solubility characteristics of PCNU mandate strict adherence to the reconstitution instructions that are provided with the drug and in the Materials and Methods section. Failure to follow this procedure will result in incomplete dissolution of the drug and may obviate the therapeutic effect.

Many of the nitrosoureas in clinical use are mutagenic, including BCNU, CCNU, methyl-CCNU, chlorozotocin, and streptozocin (17). Similar activity has not been previously reported for PCNU, but our observation of base-substitution mutagenic activity for this drug is hardly unexpected. On a quantitative basis, the mutagenic activity of PCNU would be comparable to the published activity of BCNU, CCNU, methyl-CCNU, or chlorozotocin, but much less than that of streptozocin. BCNU, CCNU, methyl-CCNU, and streptozocin are carcinogenic in animals (18), and the correlation of mutagenicity and carcinogenicity is fairly strong (15,16). These observations indicate that all nitrosoureas should be used with the understanding that second neoplasms may be induced, and PCNU is no exception. The incorporation of these agents into adjuvant programs must be undertaken with due caution.

Continued active preclinical development within the nitrosourea series has produced a sequence of promising new agents that have entered clinical trials. This process is still ongoing, as evidenced by reports of significant activity with MCNU (19) and RFCNU (20), two compounds that will soon be evaluated for clinical activity. Special characteristics of PCNU include relatively mild nausea and vomiting, lack of major nonhematologic toxicity, and animal data suggesting good penetration to CNS sanctuaries. Phase II trials of PCNU in a variety of human neoplasms will be undertaken in the near future.

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# Phase I Clinical Study of Dihydroxyanthracenedione Administered on a 5-Day Iv Schedule [1,2]

**Manuel Valdivieso,* Agop Y. Bedikian, Michael A. Burgess, Niramol Savaraj, Wanda B. Jeffers, and Gerald P. Bodey [3,4]**

## SUMMARY

**Dihydroxyanthracenedione is a new anthraquinone derivative with a wide spectrum of antitumor activity in experimental systems. A phase I clinical study was conducted using a 5-day iv schedule repeated every 3–4 weeks. Thirty-three adults with advanced refractory solid tumors received dihydroxyanthracenedione at daily doses ranging from 0.5 to 4 mg/m². Neutropenia and, to a lesser degree, thrombocytopenia were dose-limiting toxic effects, although they were of brief duration. Other toxic effects were negligible. There were no complete or partial remissions in this study. We recommend that phase II studies of dihydroxyanthracenedione on a 5-day schedule use an initial daily dose of 4 mg/m² for good-risk patients or 2–3 mg/m² for poor-risk patients. Treatment courses can be repeated at 4-week intervals.**

[Cancer Treat Rep 65:841–844, 1981]

Dihydroxyanthracenedione is one of several anthraquinone compounds which binds to DNA and inhibits nucleic acid synthesis (1). The potential importance of this compound derives from the suggestion that the cardiac toxicity of the anthracycline antibiotics was produced by the amino sugar portion of their molecule (2). Therefore, it was postulated that replacement of the amino sugar moiety by a properly selected amino or alkylamino-substituted side chain could eliminate the troublesome toxicity. Dihydroxyanthracenedione met this requirement and, in addition, it had a wide spectrum of experimental antitumor activity similar to that of doxorubicin, except that it appeared to be more active against the doxorubicin-resistant L1210 and P388 mouse leukemias, colon tumor 26, and B16 melanoma. Dihydroxyanthracenedione, however, was less active than doxorubicin against the Ridgway osteogenic sarcoma. Dihydroxyanthracenedione was active in a variety of treatment schedules and had no obvious schedule dependency (3–5).[5]

Experimental ante-mortem and post-mortem studies revealed a variety of toxic manifestations affecting primarily the hematologic and gastrointestinal systems. In beagle dogs and cynomolgus monkeys, dose-related myelosuppression and bone marrow hypoplasia were observed, with greater effect on neutrophils than on lymphocytes, platelets, and rbcs. Other side effects included salivation, emesis, diarrhea, weakness, swollen limbs, labored breathing, bluish discoloration of organs, and elevations of BUN, serum creatinine, creatinine phosphokinase, alkaline phosphatase, blood glucose, cholesterol, and triglycerides. Beagle dogs were the most sensitive large animals. Drug-related cardiac toxicity was not observed.[5]

The above information suggested that dihydroxy-

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[3]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4]We acknowledge the assistance of Mary Silverstein in the preparation of this manuscript.

**Reprint requests to:* Dr. Manuel Valdivieso, Thoracic Oncology, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

[5]Clinical brochure: anthracenedione (CL 232,315; NSC 301739). Prepared by the National Cancer Institute, Bethesda, MD, Mar 1979.

anthracenedione would be a potentially useful antitumor agent for study in man. Therefore, a phase I clinical study was initiated.

## PATIENTS AND METHODS

Adults with advanced solid tumors refractory to accepted treatment modalities were entered in this study. Presence of measurable lesions was not a prerequisite for patient entry. Patients were required to have an estimated life expectancy of at least 12 weeks and to have adequate hepatic function (normal serum bilirubin and elevation of SGOT and alkaline phosphatases to less than twice their normal values), renal function (normal BUN and serum creatinine), and adequate hematologic status (wbc counts > 3000/mm$^3$, with normal differentials, and platelet counts > 100,000/mm$^3$). In addition, patients should have recovered completely from toxic effects related to previous treatments. Patients were aware of the investigational nature of the study, and signed informed consents were obtained according to institutional policies.

Dihydroxyanthracenedione was obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, as a sterile solution containing 0.5 mg/ml of the free base of anthracenedione in 5- and 10-ml ampules. Dihydroxyanthracenedione was administered in a 5-day iv schedule repeated every 3–4 weeks, depending on the patient's recovery from toxic effects associated with the preceding course. Each daily dose was dissolved in 100 ml of 5% dextrose solution in normal saline and was administered over 30 minutes. Because of the availability of phase I clinical data on a single iv dose schedule (6), we chose an initial daily dose of 0.5 mg/m$^2$ which was equivalent to twice the usual recommended initial dose for phase I studies (one third of the toxic dose low of the most sensitive large animal). Three patients received two courses of therapy at the same dose to determine whether serious cumulative toxicity would occur. Thereafter, dihydroxyanthracenedione was escalated by 100% as long as no toxic effects were observed. After toxic effects became apparent, the dose of anthracenedione was escalated by a factor of 50%, or less if necessary. Dose escalation within patients was allowed.

Patients were closely observed during the period of drug administration and between treatment courses for as long as they remained in the study. All side effects were recorded and analyzed. Cbcs were performed twice weekly, whereas urinalysis, serum electrolytes, and SMA-100 determinations were done once weekly. Tumor measurements were recorded before each treatment course and patient response to dihydroxyanthracenedione was assessed according to standard criteria. Patients were considered to have achieved a complete remission when all symptoms and signs of cancer had disappeared. Patients were considered to have achieved a partial remission if there was a ≥ 50% reduction in the sum of the products of the two largest diameters of all measurable lesions. Patients were considered to have achieved stabilization of disease if a tumor which was rapidly growing prior to therapy showed no apparent progression for at least 8 weeks, if there was tumor response which was less than a partial remission, or if minimal progression of < 25% occurred. Patients were considered to have disease progression if there was an unequivocal increase by > 25% in the size of any measurable lesion or if new lesions developed.

## RESULTS

Thirty-three adults (24 males and nine females) with advanced malignant solid tumors were entered in this study. Their median age was 53 years (range, 23–74). All patients had evidence of tumor progression with other chemotherapeutic regimens, and 25 (77%) patients had received doxorubicin. Previous therapy included surgery in 17 patients and radiation therapy in 15 patients. Patient diagnoses are shown in table 1.

The number of patients treated and the number of treatment courses given at each dose level of dihydroxyanthracenedione are shown in table 2.

TABLE 1.—Patient diagnosis

| Diagnosis | No. of patients |
|---|---|
| Lung | |
| Adenocarcinoma | 5 |
| Squamous cell carcinoma | 4 |
| Oat cell carcinoma | 3 |
| Colon adenocarcinoma | 5 |
| Sarcoma | 4 |
| Head and neck | |
| Squamous cell carcinoma | 1 |
| Adenoid cystic carcinoma | 1 |
| Breast adenocarcinoma | 2 |
| Fallopian tube adenocarcinoma | 1 |
| Hepatocellular carcinoma | 1 |
| Renal carcinoma | 1 |
| Malignant melanoma | 2 |
| Unknown primary, adenocarcinoma | 1 |
| Medullary thyroid carcinoma | 1 |
| Embryonal carcinoma, testis | 1 |

TABLE 2.—Relationship between dose and treatment courses

| Dose | No. of patients | No. of courses |
|---|---|---|
| mg/m²/day × 5 | | |
| 0.5 | 4 | 6 |
| 1.0–1.5* | 4 | 8 |
| 2 | 6 | 12 |
| 3 | 10 | 29 |
| 4 | 5 | 11 |
| mg/m²/day × 1† | | |
| 10 | 2 | 6 |
| 12 | 2 | 2 |

*2 patients who received 2 courses each at 1.5 mg/m²/day × 5.

†Corresponded to 4 patients who received single-dose treatment during pharmacokinetic studies.

Twenty-nine patients received 66 treatment courses at dose levels ranging from 0.5 to 4 mg/m²/day × 5. For the purpose of completing an ongoing study of pharmacokinetics of this compound, the remaining four patients received eight single-dose treatments at total doses of 10–12 mg/m². This study will be reported separately.

The myelosuppressive toxicity of dihydroxyanthracenedione is shown in table 3. Myelosuppression, predominantly in the form of neutropenia, was dose-related and became dose-limiting at daily doses of 3 and 4 mg/m². Patients with extensive prior chemotherapy did not tolerate daily doses > 3 mg/m², whereas patients with prior therapy that was less extensive and did not include nitrosoureas, mitomycin, and irradiation could tolerate daily doses of 4 mg/m². The lowest blood cell counts occurred at variable times, although they occurred by the fourth week at the higher doses. Twenty-six subsequent courses at daily doses ranging between 2 and 4 mg/m² could be evaluated for evidence of cumulative hematologic toxicity. When comparing results of the first course of treatment with those of subsequent courses, we observed that the median lowest absolute neutrophil counts varied from 1.2 to 0.6 × $10^3$/mm³, whereas the median lowest platelet counts were similar, 129 and 133 × $10^3$/mm³, respectively. At doses of 3–4 mg/m², most repeat treatments could not be given before Day 28 of the preceding treatment course.

Other manifestations of drug toxicity are summarized in table 4. Although somewhat more frequent at the higher doses, they were not clearly dose-related. Mild nausea and vomiting, which were seen in approximately one third of the patients, were most common. Other toxic effects were negligible. Two patients noticed transient asymptomatic bluish discoloration of urine during the 5 days of therapy. Biochemical alterations attributable to dihydroxyanthracenedione therapy were not observed.

The antitumor effect of dihydroxyanthracenedione could be assessed in 29 patients who received at least one course of therapy and were observed for 2 weeks thereafter. The remaining four patients died during the first week of treatment because of complications of their disease and were considered to be early deaths. There were no complete or partial responders. Five patients achieved disease stabilization and 24 had tumor progression.

TABLE 3.—Myelosuppressive toxicity

| Dose | No. of courses | Median nadir counts × $10^3$ cells/mm³ (range) | | | Days |
|---|---|---|---|---|---|
| | | Wbcs | Neutrophils | Platelets | |
| mg/m²/day × 5 | | | | | |
| 0.5 | 4 | 7.8 (3.3–8.4) | 5.0 (3.1–7.1) | 271 (49*–300) | 6–16 |
| 1.0–1.5 | 7 | 3.3 (1.5–9.0) | 2.5 (1.0–7.1) | 148 (80–343) | 7–21 |
| 2.0 | 9 | 2.7 (1.0–4.6) | 1.4 (0.2–1.7) | 215 (27–385) | 8–21 |
| 3.0 | 20 | 2.2 (0.8–4.3) | 0.7 (0.0–2.9) | 115 (57–559) | 6–31 |
| 4.0 | 11 | 2.6 (0.2–4.9) | 0.8 (0.1–1.8) | 155 (20–282) | 7–24 |
| mg/m²/day × 1† | | | | | |
| 10 | 5 | 2.6 (0.8–3.5) | 2.0 (0.3–3.2) | 91 (55–472) | 10–21 |
| 12 | 2 | 1.4–5.1 | 0.9–2.8 | 81–"OK" | 11–25 |

*In a patient with squamous cell carcinoma of the lung who developed obstructive jaundice, massive progression of disease, and pneumonia.

†Corresponded to 4 patients who received single-dose treatments during pharmacokinetic studies.

TABLE 4.—Other toxic effects

| | Dose (mg/m²/day × 5) | | | | |
|---|---|---|---|---|---|
| | 0.5 | 1.0–1.5 | 2 | 3 | 4 |
| No. of courses evaluable | 6 | 7 | 9 | 18 | 9 |
| No. of courses with toxicity | 2 | 3 | 8 | 9 | 5 |
| Nausea and vomiting | 1 | 3 | 7 | 5 | 3 |
| Stomatitis | — | — | 3 | 2 | 1 |
| Fever* | — | — | 1 | 2 | 2 |
| Chest pain (?) | — | — | 1 | — | — |
| Anorexia | — | — | — | 2 | 1 |
| Myalgia | — | — | — | 1 | — |
| Constipation | — | — | — | 1 | — |
| Malaise | — | — | — | 1 | — |

*2 episodes (1 each at 2 and 4 mg/m²) associated with neutropenia, and 3 with drug administration.

## DISCUSSION

This phase I clinical study has identified that dihydroxyanthracenedione has tolerable toxic effects and can be given safely to man on a 5-day schedule, repeated every 4 weeks. Myelosuppression, predominantly in the form of neutropenia and, to a lesser degree, thrombocytopenia was dose-limiting, with some suggestion that it could be cumulative for neutropenia with repeated treatments. Other manifestations of drug toxicity were negligible.

For the most part, the toxic effects observed in this study were similar to those previously reported with a single iv dose schedule (6), although the recommended total phase II dose per course in the 5-day schedule appeared larger (20 mg/m²) than the recommended dose for single injections (12 mg/m²). This observation, however, did not result in a greater antitumor effect since there were no remissions in this study.

The lack of observed antitumor activity of dihydroxyanthracenedione in this study should not preclude the initiation of disease-oriented phase II trials with this compound in the future. The evaluation of the activity of this agent in the present study was limited by its phase I nature, in that most patients had extensive prior chemotherapy and were given treatments with inadequate doses. The marked spectrum of antitumor activity of this compound in experimental systems, coupled with its excellent tolerance in man, suggest that further studies with dihydroxyanthracenedione are indicated. We recommend that phase II trials of this agent on a 5-day iv schedule be conducted at an initial daily dose of 4 mg/m² for patients with minimal or no prior chemotherapy (good-risk patients) as opposed to a daily dose of 2–3 mg/m² for patients with extensive prior chemotherapy or radiation therapy (poor-risk patients). Treatment courses can be repeated every 28 days.

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# In Vivo Cell Cycle Phase-Preferential Killing of Murine Fibrosarcoma Cells by Cisplatin[1,2,3,4]

**Curtis P. Sigdestad,* and David J. Grdina[5,6]**

## SUMMARY

**Fibrosarcoma cells were separated by centrifugal elutriation. Selected populations enriched in $G_1$-, S-, or $G_2$ + M-phase cells were then injected ($10^4$ cells) into the tail veins of C3Hf/Kam mice. Twenty minutes later, 4 mg/kg of cisplatin was injected iv into one half of the animals in each experimental group. Fourteen days later the number of lung colonies was scored, and the surviving fractions were plotted against the elutriator fraction numbers. The results indicated that all phases of the cell cycle were sensitive to cisplatin but that the $G_1$ phase was the most sensitive by a factor of ten. Biphasic dose-response curves were observed for unseparated control, S-phase, and $G_2$ + M-phase tumor cell populations, indicating a resistant cell population. $G_1$ cells, however, had log killing over four decades without the resistant tail. Using this in vivo system, it is concluded that cisplatin kills cells throughout the cell cycle but exhibits preferential killing of $G_1$ tumor cells.**

[Cancer Treat Rep 65:845–851, 1981]

Platinum-coordinated compounds have received considerable attention (1) as antineoplastic agents since they were first introduced in 1965 (2). Many compounds have been tested for their cytotoxicity, the most active being cisplatin. It has been used effectively in the clinic against various tumors (3–5), with some nephrotoxicity noted (6). The side effects have been ameliorated to some extent by hydration and the use of diuretics (7).

For many anticancer drugs, the killing effects on tumor cells depend on the position of the cells in the cell cycle. The purpose of the present study was to characterize the age-related response of fibrosarcoma (FSa) cells lodged in the lungs of C3H mice to treatment with cisplatin. In addition, the in vivo dose response of selected tumor populations to cisplatin was determined.

## MATERIALS AND METHODS

### Tumor System

Male C3Hf/Kam mice, 9–10 weeks old, and a methylcholanthrene-induced FSa were used in this study. Source tumors were grown in both hind legs of the animals following the im injection of $5 \times 10^5$ viable tumor cells. Tumors, having an average diameter of 10–12 mm, were excised and made into a single-cell suspension by mincing and trypsinization (8). Cell viability was determined by trypan blue exclusion with phase-contrast microscopy and was routinely > 95%. To reduce tumor cell heterogeneity and better prepare the cells for elutriation,

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[5]Section of Experimental Radiotherapy, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

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**Reprint requests to:* Curtis P. Sigdestad, PhD, Therapeutic Radiology Department, University of Louisville, Radiation Center, 500 S Floyd St, Louisville, KY 40292.

the cell suspension was incubated in vitro for 48 hours (9). Primary cultures were made by seeding $1.5 \times 10^7$ viable cells in 32-ounce glass culture bottles (20 bottles/experiment) and incubating at 37°C in a water-saturated atmosphere of 5% $CO_2$ and air. The growth medium was a modified McCoy's 5A medium supplemented with 20% fetal calf serum (10).

### Cell Separation

FSa cells ($3 \times 10^8$ in exponential growth) were separated at 4°C by centrifugal elutriation using a Beckman JE-6 centrifugal elutriator rotor (Beckman Instruments, Inc, Cedar Grove, NJ) (11). The system was sterilized with 70% ethanol (12). The elutriation separation was accomplished using a modified McCoy's 5A medium supplemented with 5% fetal calf serum. Also included was DNase (Sigma Chemical Co, St. Louis, MO) at a final concentration of 0.1 mg/ml. Centrifugation speed was kept constant (1200 rpm) and medium flow rates were increased by increments of 2 ml/minute. In this manner, 12 fractions of 50 ml each were routinely collected.

Cells in each fraction were characterized with respect to viability, using phase-contrast microscopy; size, using a model ZBI Coulter counter and multichannel analyzer (Channelyzer II, Coulter Electronics); and relative DNA content, using an ICP21 flow microfluorometer (Phywe Co, Göttingen, W Germany). For the latter assay, aliquots of cells from each fraction were stained with mithramycin, a DNA-specific fluorescent dye, and the resulting flow microfluorometer profiles were computer-analyzed (13).

### Lung Colony Assay

Mice, with hind legs shielded, were given whole-body irradiation with 1000 rad. Twenty-four hours later these animals were injected with $10^4$ viable FSa cells from each of the elutriator fractions along with $10^6$ heavily irradiated (10,000 rad) FSa cells. This was done to maximize the efficiency of lung colony formation in these animals (14). After 14 days, the mice were killed, the lungs were removed and fixed in Bouin's solution, and the colonies were counted.

### Cisplatin Testing In Vivo

Cisplatin was diluted initially with sterile water. Additional dilutions were made with sterile saline to adjust the concentration to reasonable injection volumes. To characterize the cytotoxic effect of cisplatin on synchronized FSa cells in vivo, the drug was injected iv 20 minutes after tumor cell injection into one half of the animals in each experimental group. Under these conditions, no drug toxicity was observed in the recipient animals.

Selected elutriator fractions were pooled to generate dose-response curves for synchronized FSa cell populations. Populations of cells enhanced in either $G_1$-, S-, or $G_2$ + M-phase were exposed to different concentrations of drug while lodged in the lungs of the test animals. Untreated control mice were compared with treated animals to calculate the percent of cells surviving.

## RESULTS

The recovery of cells following centrifugal elutriation was routinely > 95%. The recovery of FSa cells and the percent of cells in the various phases of the cell cycle for each of the elutriator fractions are presented in figure 1 for comparison. No normal diploid cells, as determined by flow microfluorometer analysis, were detected in any of the recovered elutriator fractions (9).

The effect of 4 mg/kg of cisplatin in vivo on the various elutriated cell fractions is described in figure 2. While cisplatin was effective at this concentration in reducing the number of lung colonies in all experimental groups by at least a factor of ten, it was most effective against the $G_1$ tumor cell populations (ie, fractions 3 and 4, having 91% and 90% $G_1$-phase cells, respectively). There was about a 100-fold reduction in lung colonies in these treated groups as compared to controls. The response of an unseparated control (USC) tumor population to cisplatin is also presented in figure 2 for comparison.

To further characterize the effectiveness of cisplatin in vivo, USC cells and selected elutriator fractions of FSa cells, designated fractions FSa:A, FSa:B, and FSa:C, were injected into mice, and dose-response curves were determined (fig 3).

The survival curves describing the responses of the USC, FSa:B, and FSa:C populations to cisplatin are all biphasic. The low-dose regions of the curves are characterized as having little or no shoulder, as well as similar $D_0$ values (ie, USC, 0.69 mg/kg; FSa:B, 0.67 mg/kg; and FSa:C, 0.62 mg/kg). The $D_0$ values describing the resistant tail region of these survival curves did vary considerably though. However, because of the relatively few survival points at that region, it is not certain whether these differences are significant. In contrast, the survival curve describing the response of the $G_1$-enhanced population (ie, FSa:A) did not exhibit a biphasic shape. Cell killing was exponential over four decades with a $D_0$ of 0.45 mg/kg.

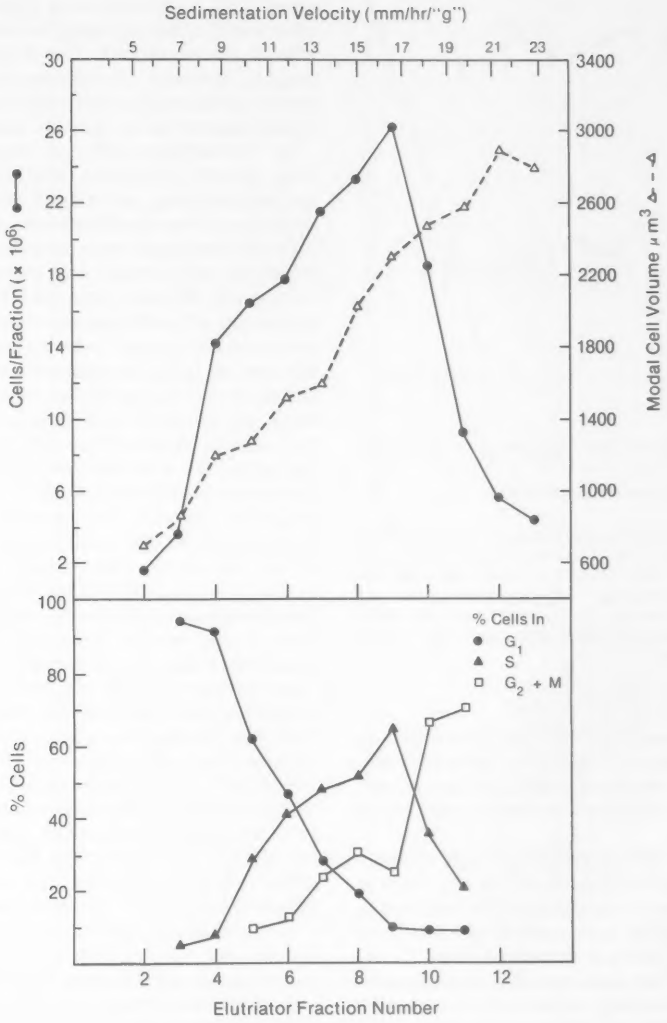
Sedimentation Velocity ( mm/hr/"g")
5 7 9 11 13 15 17 19 21 23
30 26 22 18 14 10 6 2
Cells/Fraction ( × 10⁶)
3400 3000 2600 2200 1800 1400 1000 600
Modal Cell Volume μm³
100 80 60 40 20
% Cells
% Cells In
G1
S
G2 + M
2 4 6 8 10 12
Elutriator Fraction Number

FIGURE 1.—The No. of FSa cells recovered (●——●), the modal cell volume (Δ- - -Δ) (top), and the percent of cells distributed among the various cell cycle phases (bottom) are plotted as a function of sedimentation velocity and elutriator fraction No.

## DISCUSSION

The success of chemotherapy in the treatment of tumors is dependent on the number of clonogenic and potentially clonogenic tumor cells that are destroyed or sterilized. The primary tumor, while usually considered as a single entity, is not a simple cell system, rather it is extremely complex and heterogeneous. Not only are tumor cells dispersed throughout the phases of the cell cycle, but they are exposed to a variety of physiologic conditions such as differences in availability of nutrients, oxygen tension, and pH. Each of these factors can, in turn, affect the response of cells within the envi-

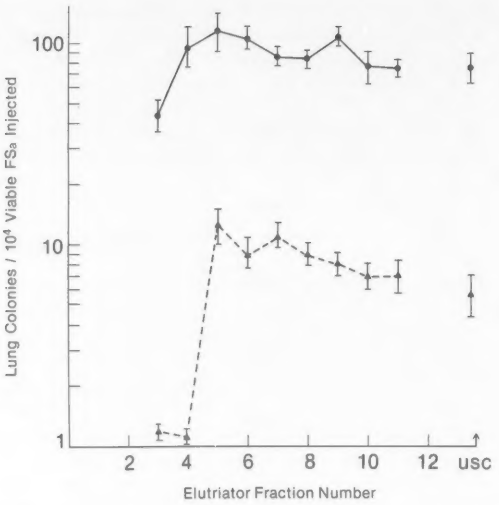


FIGURE 2.—The effect of cisplatin on the ability of synchronized FSa cells to form lung colonies. The No. of lung colonies formed per $10^4$ viable tumor cells injected in control (●——●) and cisplatin-exposed (4 mg/kg) (▲- - -▲) animals are plotted as a function of elutriator fraction No. Vertical bars = SE. Refer to bottom panel of figure 1 for distribution of cells in the various phases of the cell cycle.

ronment of the tumor and thus exert an effect on the ability of the tumor to respond to chemotherapy. The present study reports the cell cycle phase-preferential killing, by cisplatin, of tumor cells lodged in the lungs.

Knowledge of the differential cytotoxicity of drugs toward cells in various phases of the cell cycle is extremely important to the design of chemotherapy protocols. Such studies have most frequently been carried out in vitro using cultured cell lines. To obtain synchronized populations, these investigators used techniques involving either mitotic selection or drugs. Each of these techniques, however, has only limited application to the in vivo situation. The mitotic shake procedure, for example, can only be applied to a relatively few cultured cell lines and cannot be applied to in vivo systems such as solid tumors. In addition, the yield of cells using this technique is usually very low. While this approach has given rise to a considerable amount of information, many difficulties exist in relating these data to the complex in vivo situation (15). In vivo studies have been performed, but they generally involve the use of two or more cytotoxic agents. A partial synchrony of "target" cells is induced by first exposing the host animal to a known phase-specific agent such as hydroxyurea (16). At varying times afterward, a second agent is administered and its effects are monitored. The difficulty with this approach, however, is in discerning whether the response of the cells to the second agent is perturbed in any manner as a result of exposure to the first.

In vivo testing of cell cycle phase cytotoxicity has been greatly simplified with the development of methods utilizing centrifugal elutriation (17). This method exploits size differences between cells. Under uniform conditions of exponential growth, cells increase in size steadily and exponentially with age during the division cycle (18). Since the sedimentation rate of a cell is proportional to the two-thirds power of its volume, velocity sedimentation methods can be used to separate and isolate subpopulations of cells having similar sizes and DNA contents from asynchronous populations. Cells enriched in the various phases of the cell cycle are injected iv into mice. At selected times later, the drug to be tested is administered either iv, ip, or sc. With appropriate controls, the number of lung colonies formed reflects the phase-preferential cytotoxicity of the test agent. This procedure is advantageous in that it is applicable to testing drugs that require bioactivation. Additionally, relatively large numbers of cells can be separated, synchronized, and recovered without loss of viability.

In the experiments reported here, whole-body-irradiated mice and heavily irradiated tumor cells were used in the lung colony procedure in order to enhance the colony-forming efficiency of the injected cells. Under these conditions, tumor cell retention and colony-forming efficiency were found to be independent of either cell size or position within the division cycle (14). This method has been used effectively to characterize in vivo the phase-specific effects of both hydroxyurea and doxorubicin (17,19).

Cell killing by cisplatin, as evidenced by a reduction in lung colony number in treated as compared to untreated animals, was found to be greatest for populations containing primarily $G_1$-phase cells, ie, elutriator fractions 3 and 4. The enhanced sensitivity of $G_1$ cells was also reflected in the dose-response curves obtained following in vivo exposure to various concentrations of drug.

Survival curves describing the response of USC, FSa:B, and FSa:C tumor populations were biphasic, indicating the presence of relatively drug-resistant cells. In contrast, the relatively steep slope and shape of the curve describing the FSa:A populations are indicative of the response of a relatively homogeneous and sensitive population of cells.

Similar results of cell cycle phase-preferential killing with cisplatin have been previously reported using in vitro models. Drewinko and Gottlieb (20)

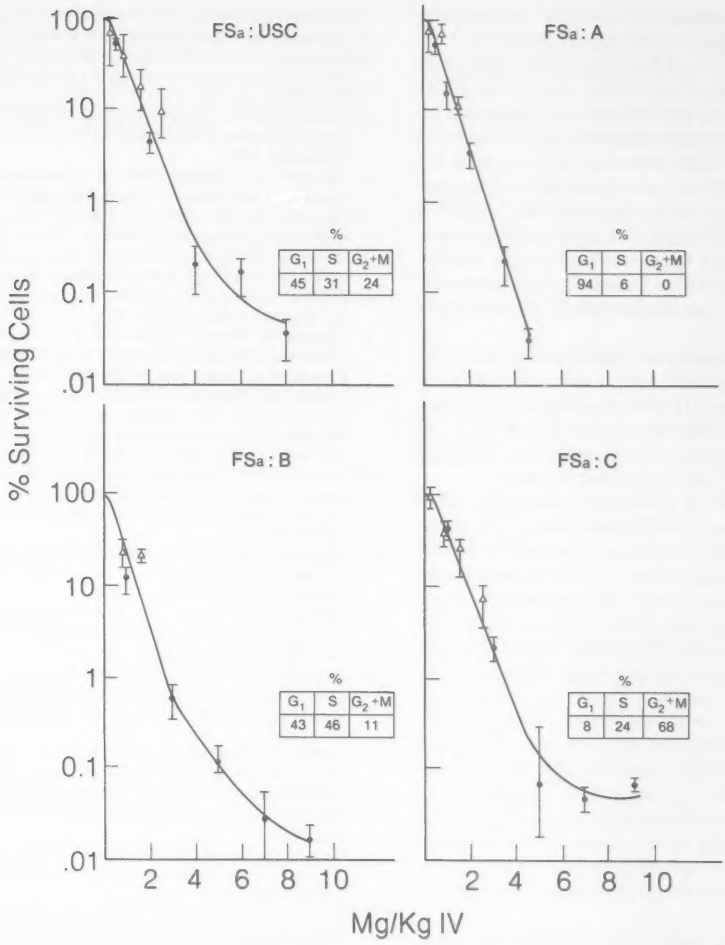


FIGURE 3.—The response of selected FSa cell populations, treated while retained in the lungs of C3Hf/Kam mice, to different doses of cisplatin. Populations are designated USC and FSa:A, FSa:B, and FSa:C for selected elutriator-separated cells. $D_0$, extrapolation No. (n), and associated SE of the mean, determined by computer analysis, are as follows: USC: $D_0$ = 0.69 (0.62, 0.76), n = 2.00 (1.42, 2.82); FSa:A: $D_0$ = 0.45 (0.42, 0.48), n = 1.41 (1.06, 1.88); FSa:B: $D_0$ = 0.67 (0.63, 0.71), n = 1.63 (1.67, 2.29); and FSa:C: $D_0$ = 0.62 (0.58, 0.65), n = 1.63 (1.30, 2.04). Vertical error bars = 1 SE of the mean. Symbols represent 2 different experiments.

tested the effects of cisplatin on lymphoma cells synchronized by excess thymidine. Their results were very similar to those reported here. The DNA synthetic phase and the $G_2$ phase of the cell cycle showed only slight variation in cell sensitivity. Cells in the $G_1$ phase, however, showed increased sensitivity to the agent. Dose-response survival curves, unlike the data reported here, all showed logarithmic killing for each cell cycle phase over the entire dose range tested. The $G_1$ cells did, however, show a slightly steeper slope, to which little significance was ascribed. Szumiel and Nias (21), studying other platinum complexes, have demonstrated no cell-age dependency for cytotoxic effects on CHO cells synchronized by mitotic selection.

Fraval and Roberts (22), using the mitotic shake method of synchronizing V79 Chinese hamster cells, also showed that $G_1$-phase cells exhibited maximum sensitivity to cisplatin. Their dose-response data, although extending only over one or two decades of

cell kill, show significant differences in both the shoulder and the slope of the survival curves.

A recent publication of Meyn et al (23) described the effects of cisplatin and other agents on CHO cells synchronized by centrifugal elutriation. The age-response curves were similar to those reported here; however, the dose-response data showed exponential killing, with $G_1$ cells showing the steepest slope. Other authors have reported that cisplatin produces DNA-to-DNA and DNA-to-protein cross-linking (24,25). These types of damage are similar to those produced by bifunctional alkylating agents, which are known to act preferentially against cells in $G_1$ phase (26).

The in vivo dose-response data reported here indicate a bimodal survival, which may indicate a resistant subpopulation. It is interesting to note that as more and more cells are in the S and $G_2$ + M phases of the cell cycle, the slope of the resistant tail of the survival curve approaches zero.

A modification of this protocol is presently under study, which may be of additional value in discerning the cell cycle effects of drug treatment in solid tumors. This modification involves the in vivo treatment of asynchronous tumor cells growing as macroscopic colonies in the lung, with subsequent isolation, elutriation, and clonogenic assay. The end results of experiments of this type should reflect cell cycle effects of the test agent on tumor cells where factors such as bioactivation, biologic half-life, differences in diffusion distances, route of injection, and metabolism may modify the cell response. In addition, the effects of multidrug or combination chemo-radiotherapy regimens can be studied. Experiments of this type are of extreme importance in the design of protocols that will be maximally effective in the eradication of the tumor cell population.

In conclusion, we have characterized the cytotoxic effectiveness of cisplatin against elutriator-synchronized populations of tumor cells lodged in the lungs of test animals. In this manner it has been demonstrated that cisplatin is effective in vivo against cells in all phases of the cell cycle, but it is most toxic to $G_1$-phase cells. The dose-response data indicate bimodal cell kill for the asynchronous population and during all phases of the cell cycle with the exception of $G_1$, during which exponential killing occurred. These latter results differ from the in vitro data reported previously (20,22,23).

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# Rescue of Thymidine Cytotoxicity in L1210 Ascites by Elevated Endogenous Levels of Deoxycytidine[1,2]

E. Michael Egan, Lalla Sargent, Andre Rosowsky, and Donald W. Kufe[3,4,*]

## SUMMARY

**Although the biochemical and cytokinetic effects of thymidine (TdR) have been extensively studied in vitro, other parameters may be encountered in vivo that could alter its therapeutic efficacy. L1210 cells in culture are sensitive to the growth inhibitory effects of TdR, while TdR infusions in mice bearing L1210 ascites fail to prolong survival. Our results demonstrate that the TdR levels achieved in the serum and ascites fluid during sc and iv infusions range between $10^{-4}$ and $10^{-3}$ M. These levels are sufficient to inhibit the growth of bone marrow and intestinal mucosa cells, as evidenced by $^{32}P$ incorporation studies. However, these TdR levels fail to inhibit the growth of L1210 ascites, despite the fact that these cells are sensitive to similar concentrations in vitro. The absence of growth inhibition in the L1210 ascites is shown to be due to increased endogenous CdR levels ($\sim 2 \times 10^{-5}$ M) in the ascites fluid. The concomitant serum CdR levels were at least ten times lower than those achieved in the ascites. This factor probably accounts for the differential effect of TdR on normal cells as compared to the L1210 cells and suggests that the antitumor response achieved in vivo with agents such as TdR or even antimetabolites, such as cytarabine, may be modulated by increased CdR levels in the local tumor milieu. These studies are relevant in that TdR is currently being used in a variety of clinical protocols using the nucleoside alone at high doses or in combination with methotrexate, 5-FU, or cytarabine.**

[Cancer Treat Rep 65:853–860, 1981]

The effects of thymidine (TdR) on mammalian cell growth in vitro have been studied extensively. Cellular growth is inhibited through regulation of several enzymes in pyrimidine biosynthesis by dTTP (1,2). An increase in dTTP levels limits the production of dCTP by inhibiting ribonucleotide reductase and thus the conversion of CDP to dCDP (3). The deficiency of dCTP limits DNA synthesis, and CdR can bypass this block to re-initiate cell growth.

TdR inhibition of cellular growth has been demonstrated in a variety of in vitro systems. In Chinese hamster and Novikoff hepatoma cells, growth is completely inhibited by exposure to 1–2-mM TdR (4–6). Studies with human cell lines have produced similar results (6–8). Lymphoblast lines of both murine and human origin are more sensitive to TdR and undergo complete inhibition of growth at concentrations of $5 \times 10^{-5}$ M (9,10).

In vivo studies indicate that TdR causes regression of human tumor xenografts in nude mice (11–

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[3]Divisions of Pharmacology and Medicine, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA.

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*Reprint requests to: Donald W. Kufe, MD, Division of Medicine, Sidney Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

13). Other investigations in animal tumor models, however, have failed to demonstrate antitumor activity. TdR has been shown to be ineffective in the L1210, Lewis lung, colon tumor 26, and colon tumor 38 transplantable murine tumors, when a low-dose intermittent schedule is used.[5] Using high-dose continuous iv infusions of TdR in doses up to 18 g/kg/day, Semon and Grindey (14) also demonstrated a lack of effect on L1210 ascites in DBA/2J mice. Investigations in our laboratory have shown that the continuous iv infusion of high doses of TdR in rats results in the arrest of myeloblastomas as well as rapidly proliferating normal tissues (15). TdR had no significant effect on myeloblast growth when it was tested against subcutaneous tumor nodules > 1 $cm^3$ or when myeloblasts were grown as a malignant ascites.

The discrepancy between in vitro sensitivity of cellular growth to TdR and the absence of significant antitumor effects with the nucleoside in animal tumor models has prompted the present investigation. This report provides data supporting the in vivo rescue of L1210 ascites from TdR growth inhibition by increased endogenous levels of CdR.

## MATERIALS AND METHODS

### TdR Infusions

$BDF_1$ mice (20 g) were obtained from Jackson Laboratories (Bar Harbor, ME). In each experiment, $10^5$ L1210 cells were inoculated ip into recipient mice, and the infusions were begun after 24 and 72 hours. The TdR infusions (72 hours) were performed either sc in the flank region or by the iv subclavian route (16). The infusion volume was 0.45 ml/hour at the dose of 900 mg/kg/hour. The median survival was calculated from the day of tumor inoculation.

TdR was obtained from the Sigma Chemical Co (St. Louis, MO). Solutions were prepared at a TdR concentration of 50 mg/ml in 0.3% (wt/vol) sodium chloride. The solutions were filtered for sterility and stored at room temperature. Drug doses were calculated on the basis of body weight. Infusions were performed with a Harvard infusion pump (model 940) with an adapter to hold eight syringes (Harvard Apparatus, Inc, Millis, MA).

### Pyrimidine Assays

TdR (17), thymine, and CdR levels were measured in serum and ascites fluid samples by high-pressure liquid chromatography (HPLC). A Waters liquid chromatograph (model AL-C202; Waters Associates, Milford, MA) equipped with a U6K injector, dual model 6000 pumps, model 600 solvent programmer, and a standard 254-nm UV absorbance detector was used. A Schoeffel Spectroflow monitor (model SF-770) was connected to the system to allow the absorbance to be monitored simultaneously at 270 nm. A Waters μ-Bondapak CN column (3.9-mm inner diameter × 30 cm) (Waters Associates) was used, with sodium chloride buffer as the eluting solvent. After addition of enough BUdR (the internal standard) to each serum and ascites specimen to give a final concentration of $2 \times 10^{-4}$ M, the proteins were removed by heat precipitation and centrifugation, and the supernatant was then filtered through a 0.22-μm Millipore filter. Samples of 1–5 μl were injected in triplicate.

### Cell Culture Studies

L1210 cells transplanted as an ascites for the in vivo studies were adapted for growth in tissue culture. The cell line was maintained in minimum essential media for suspension supplemented with 10% heat-inactivated fetal calf serum, 100 units of streptomycin/ml, 100 μg of penicillin/ml, 1% L-glutamine, and 0.05% β-mercaptoethanol. Single-cell suspensions were inoculated into Linbro multi-well tissue culture plates (Linbro Scientific Products, Hamden, CT) and were maintained in log phase growth at the time of drug exposure. TdR and CdR (Sigma Chemical Co) were freshly prepared in complete medium at concentrations of $10^{-2}$ M. The stock solutions were sterilized by Millipore filtration, and serial dilutions were made to give final TdR concentrations of $10^{-2}$ to $10^{-7}$ M and final CdR concentrations of $10^{-4}$ to $10^{-6}$ M. Cells were exposed to various concentrations of the nucleosides for 72 hours, harvested, and counted in a model Z Coulter counter. Cell viability results are expressed as the percent of growth inhibition in comparison with parallel control cultures that were treated similarly without the addition of nucleosides. Values represent the mean of four determinations repeated three times.

### Cell Kinetic Studies

The in vivo labeling studies were performed by ip injection of 250 microcuries of $^{32}P$, carrier free (New England Nuclear Corp, Boston, MA). After appropriate time intervals, animals were killed for harvesting of the L1210 ascites, bone marrow, and intestinal mucosa cells (15). The cells were washed three times with phosphate-buffered saline and re-

[5]Clinical brochure: thymidine. Prepared by the National Cancer Institute, Bethesda, MD, Oct 1978.

TABLE 1.—The effect of TdR infusions on survival of mice bearing L1210 ascites*

| Dose (mg/kg/hr) | Survival: Range (days) | Survival: Median (days) | % ILS |
|---|---|---|---|
| Control | 9–11 | 9 | — |
| 300 | 9–11 | 9 | 0 |
| 600 | 9–11 | 11 | +22 |
| 900 | 9–11 | 10 | +11 |
| 1200 | 2–11 | 6 | –33 |
| 1800 | 2 | 2 | –78 |

*$BDF_1$ (7/group) were inoculated ip with $10^5$ L1210 cells. TdR was given sc by continuous infusion at various doses. Control mice were infused in a similar manner with saline.

Infusions were begun 72 hrs after tumor injection and were continued for 72 hrs. The % increase in lifespan (ILS) was calculated according to the equation: % ILS = 100 (t/c – 1), where t is the median survival time of the treatment group in days and c is the median survival time of the control group. Increased survival times obtained with infusions of 600 and 900 mg/kg/hr were not statistically significant.

suspended in phosphate-buffered saline at a final concentration of $10^6$ cells/ml. The incorporation of $^{32}P$ into DNA was determined after alkali digestion, as previously described (15). Results are corrected for incorporation per microgram of total cellular DNA, as determined by the Burton method (18).

## RESULTS

### Animal Survival Studies

Table 1 shows the effect of TdR infusions on survival. Control animals were infused with a volume of saline equivalent to that given to animals at the highest TdR dose. There was no difference in the overall survival (median, 9 days) of animals that received saline infusions and that of uninfused tumor-bearing animals. TdR doses were increased from 300 to 1800 mg/kg/hour. Early toxic deaths were observed at doses of 1200 and 1800 mg/kg/hour and resulted in a decrease in lifespan for both groups. Infusions at doses of 600 and 900 mg/kg/hour resulted in minimal increases in survival that were not statistically significant. Similar results were achieved when the infusions were begun either 24 or 72 hours after tumor inoculation.

### Cell Kinetic Studies

The absence of a significant increase in survival suggested that these TdR infusions had no effect on L1210 cell growth in vivo. The effect of TdR on cell cycle traverse in vivo or in vitro can be monitored by following the incorporation of $^{32}P$ into cellular DNA. Figure 1 shows the relative rates of DNA synthesis as determined by $^{32}P$ incorporation in the L1210 ascites (A), bone marrow (B), and intestinal mucosa (C) during infusions of either saline or TdR. TdR was administered at the highest nontoxic dose of 900 mg/kg/hour for a period of 72 hours prior to administration of $^{32}P$. Figure 1A shows that $^{32}P$ incorporation is similar in the L1210 ascites for animals infused with either saline or TdR and is consistent with that expected from the survival data. In contrast, DNA synthesis was inhibited in the bone marrow (fig 1B) and intestinal mucosa (fig 1C) preparations as a result of the TdR infusions. Thus, in each normal tissue, the $^{32}P$ incorporation after 4 hours of labeling was four to six times less following TdR exposure than in the tissue of the controls.

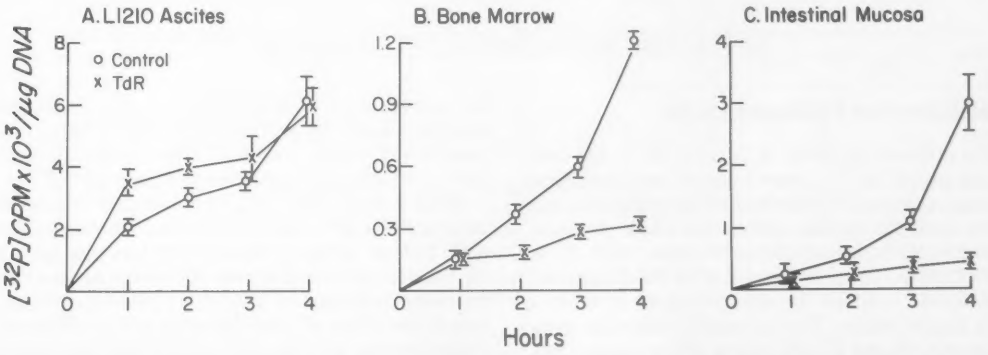


FIGURE 1.—Relative rates of DNA synthesis as determined by $^{32}P$ incorporation/microgram of DNA isolated from L1210 ascites, bone marrow, and intestinal mucosa over a 4-hr period during iv infusions of either saline (○) or TdR (X) at a dose of 900 mg/kg/hr in vivo.

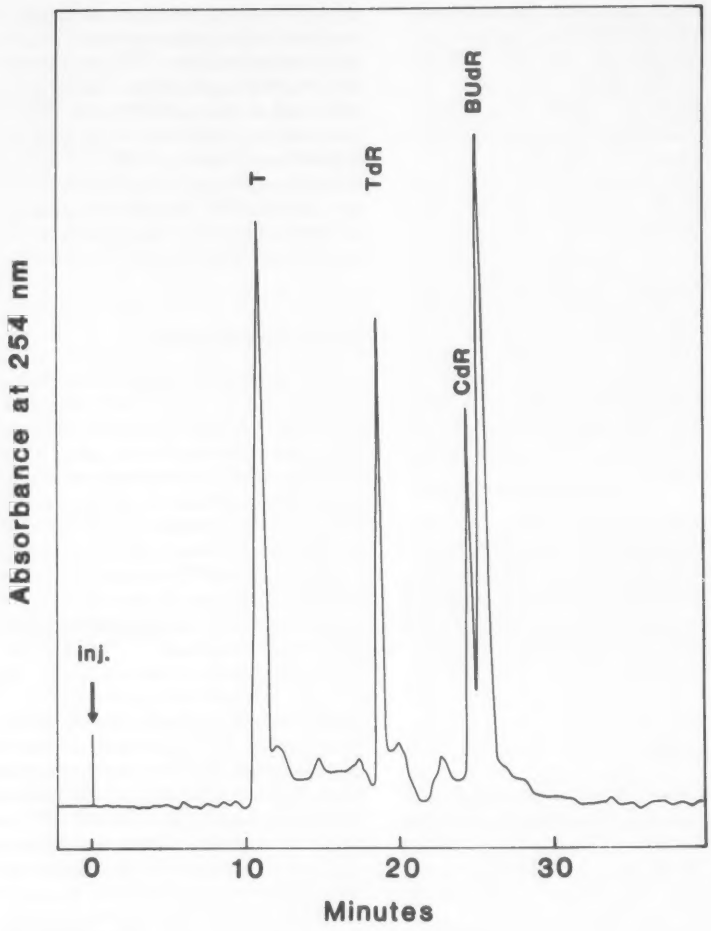


FIGURE 2.—HPLC separation of thymine, TdR, CdR, and BUdR.

## Measurement of Pyrimidine Levels

The differential effect of TdR on the L1210 cells as compared to the bone marrow and intestinal mucosa prompted further studies on pyrimidine levels in both the serum and ascites fluid. A representative HPLC separation of thymine, TdR, CdR, and BUdR is shown in figure 2. The BUdR internal standard is included for determination of relative peak height ratios. The incomplete baseline separation of CdR and BUdR did not affect these ratios, and the CdR levels were confirmed in the absence of the internal standard. The CdR peak was identified both by 254/280-nm UV wavelength ratios and by co-migration. The 254/280-nm ratio for a CdR standard was 0.94, and the ratio for an ascites fluid sample CdR peak was 0.96. The co-migration results for CdR are illustrated in figure 3A–C.

Table 2 shows the TdR, thymine, and CdR levels measured by HPLC for controls and animals infused with TdR at nontoxic doses. TdR levels of $10^{-4}$ to $10^{-3}$ M were achieved in both the serum and ascites, following infusions of 300–900 mg/kg/hour. Variation in the route of administration of the nucleoside yielded similar results, although the TdR levels were generally higher with iv infusions. Thymine, a catabolic product of TdR, was also elevated in animals infused with the nucleoside, as shown in table 2.

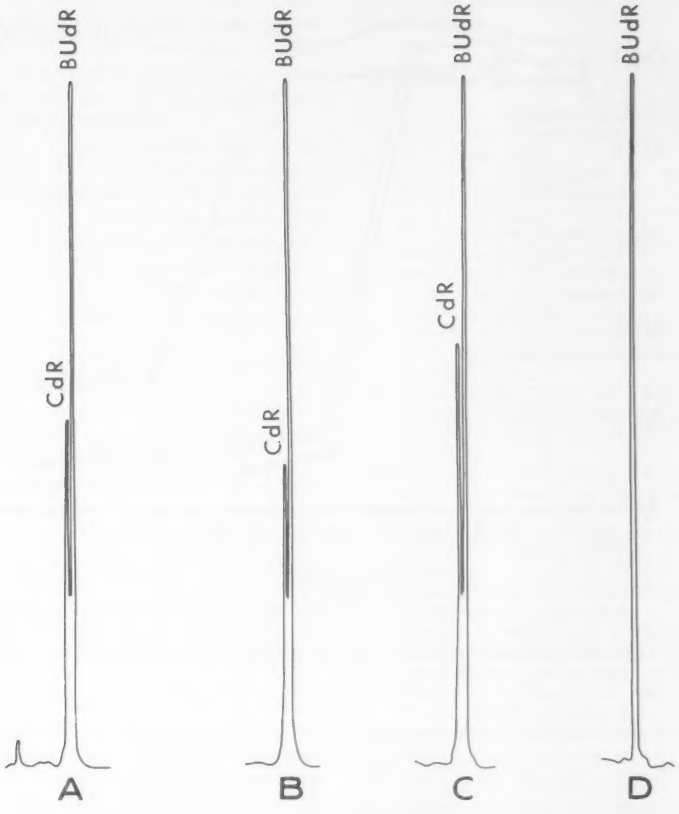


FIGURE 3.—HPLC separation of CdR and BUdR: (A) standard solution of CdR and BUdR; (B) ascites fluid sample; (C) ascites fluid sample with CdR standard added; and (D) incubation of ascites fluid or $10^{-5}$ M CdR standard with $BDF_1$ plasma for 15 mins at 37°C.

TABLE 2.—Serum and ascites fluid pyrimidine concentrations ($10^{-4}$ M) in L1210-bearing mice infused with saline or TdR*

| Infusion (mg/kg/hr) | TdR | | Thymine | | CdR | |
|---|---|---|---|---|---|---|
| | Serum | Ascites | Serum | Ascites | Serum | Ascites |
| Saline | 0.064 ± 0.001 | 0.077 ± 0.003 | 0.071 ± 0.002 | 0.074 ± 0.007 | ND | 0.11 ± 0.04 |
| TdR (sc) | | | | | | |
| 300 | 0.74 ± 0.12 | 0.93 ± 0.09 | 0.44 ± 0.07 | 0.58 ± 0.07 | ND | 0.13 ± 0.12 |
| 600 | 4.6 ± 1.9 | 1.1 ± 0.8 | 1.1 ± 2.7 | 1.7 ± 0.6 | ND | 0.17 ± 0.16 |
| 900 | 6.4 ± 0.4 | 4.4 ± 0.2 | 1.1 ± 0.7 | 1.5 ± 1.3 | ND | 0.14 ± 0.13 |
| TdR (iv) | | | | | | |
| 300 | 3.0 ± 1.2 | 3.2 ± 0.5 | 0.60 ± 0.01 | 0.82 ± 0.03 | ND | 0.08 ± 0.07 |
| 600 | 5.3 ± 2.7 | 5.8 ± 3.6 | 0.98 ± 0.02 | 1.15 ± 0.16 | ND | 0.19 ± 0.18 |
| 900 | 13.0 ± 1.6 | 8.2 ± 0.9 | — | — | — | — |

*$BDF_1$ mice (4/group) were inoculated with $10^5$ L1210 cells. TdR was administered sc or iv by continuous infusion for 72 hrs 3 days following tumor injection. Control mice were given saline infusions in a similar manner. Serum and ascites fluid samples were obtained just prior to completion of the infusion and the levels were measured by HPLC. Results = mean $10^{-4}$ M concentration ± SE. ND = not detectable ($< 1 \times 10^{-6}$ M).

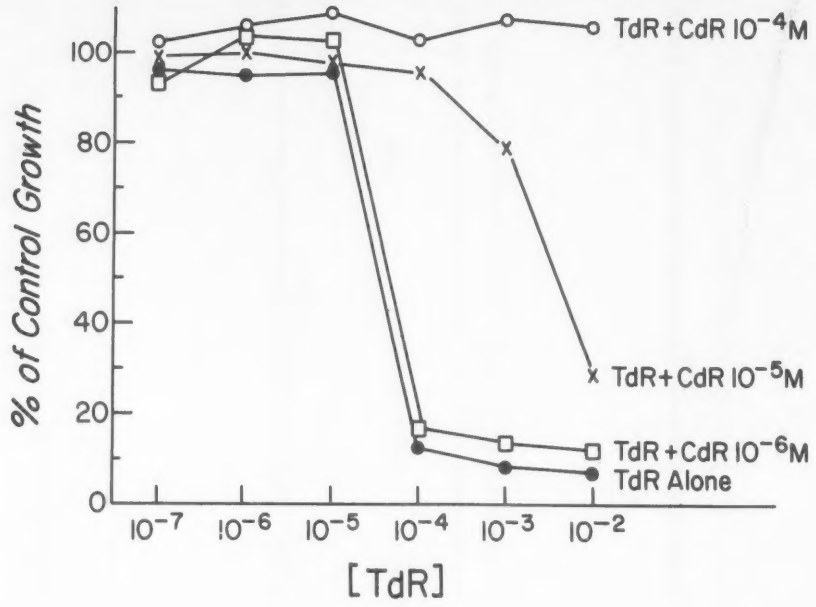


FIGURE 4.—Growth inhibition of L1210 cells 72 hrs after continuous exposure to various concentrations of TdR and CdR in vitro.

The lower limit of detectability for the measurement of CdR was $10^{-6}$ M. Serum samples from control animals and animals infused with TdR had undetectable CdR levels, as listed in table 2. In contrast, the ascites fluid contained CdR at significant levels > $10^{-5}$ M in both of the control animals, as well as in those infused with TdR. When either ascites fluid or a $10^{-5}$ M CdR standard was incubated with equal volumes of fresh $BDF_1$ mouse plasma for 15 minutes at 37°C, the CdR peaks were reduced to a level below detectability (fig 3D).

### In Vitro Studies

The L1210 cells used in the in vivo studies were grown in tissue culture for two to ten passages to determine the effect of variations in TdR and CdR concentrations on cell growth. As shown in figure 4, the TdR concentrations ranged from $10^{-7}$ to $10^{-2}$ M, and after 72 hours of exposure, growth inhibition was nearly complete at concentrations ≥ $10^{-4}$ M. The concomitant addition of CdR at concentrations of $10^{-6}$ M yielded similar results, while CdR at a concentration of $10^{-5}$ M achieved a partial reversal of the growth inhibition. A complete rescue from the effects of TdR was achieved with CdR at concentrations of ≥ $10^{-4}$ M. CdR alone had no effect on cell growth when it was tested at levels of up to $10^{-3}$ M.

## DISCUSSION

TdR is currently being employed in a variety of clinical protocols using the nucleoside alone in high doses or in combination with certain antimetabolites such as methotrexate, 5-FU, or cytarabine. Although the biochemical and cytokinetic effects of TdR have been extensively studied in vitro, other parameters may be encountered in vivo that could alter its therapeutic efficacy. In vitro studies have demonstrated cellular sensitivity to TdR in a variety of mammalian tumor lines, while responses in the corresponding animal model or in clinical studies have been more variable (11–13, 19–21).

TdR-induced cell cycle traverse arrest has been reversed in both murine and human cell lines by the addition of CdR (22,23). Thus, high serum levels of CdR could provide a potential mechanism for in vivo TdR resistance. Plasma CdR levels of $2 \times 10^{-5}$ M have been demonstrated in the rat, while levels of approximately $10^{-7}$ M have been reported in mice (24). Our results in the $BDF_1$ mouse indicate serum

CdR levels < $10^{-6}$ M. However, in contrast, similar measurements in the L1210 ascites have demonstrated CdR levels > $10^{-5}$ M. The reason for this high endogenous CdR level in the ascites remains unclear, but it could be due to cellular degradation with release of metabolic products.

As evidenced by the tissue culture studies, the growth of the L1210 cells used in the in vivo studies is inhibited by concentrations of TdR in excess of $10^{-5}$ M. The TdR levels achieved in ascites fluid during the sc and iv infusions ranged between $10^{-4}$ and $10^{-3}$ M. L1210 growth in vitro was inhibited by > 90% with exposure to these concentrations for 72 hours. The addition of CdR at a concentration of $10^{-6}$ M to cells concurrently exposed to TdR at concentrations > $10^{-4}$ M failed to reverse the growth inhibition, while a CdR concentration of $10^{-5}$ M provided nearly complete rescue. The concentration of CdR ($\sim 2 \times 10^{-5}$ M) in the ascites fluid is, therefore, sufficient to reverse the growth inhibition expected from the TdR levels achieved during these infusions.

The cell kinetic studies which monitor DNA synthetic activity by incorporation of $^{32}P$ confirm the absence of a TdR-induced inhibition of the L1210 cell growth. In contrast, bone marrow and intestinal mucosa cell growth is inhibited in the animals infused with TdR. The exposure of these tissues to CdR is less than that of the L1210 cells, as the serum CdR levels are at least ten times lower than those achieved in the ascites. Incubation of the ascites fluid or of a CdR standard ($10^{-5}$ M) with $BDF_1$ serum resulted in the rapid degradation of this nucleoside. This effect is probably due to CdR deaminase in the plasma[6] and would account for the differential effect of TdR on bone marrow and intestinal mucosa cells as compared to L1210 ascites cells.

The antitumor response achieved in vivo with agents such as TdR may, therefore, be modulated by increased levels of certain metabolites, either in the local tumor milieu or in the serum. The increased CdR levels in the L1210 ascites reverse the effect of TdR and may alter the efficacy of antimetabolites such as cytarabine, which is relatively ineffective when tested against the L1210 animal tumor model (25) but is very effective in the L1210 tissue culture system (26).

Although CdR was monitored in these studies, localized elevations may pertain as well to the purine nucleosides. Finally, it is certainly relevant to consider local and systemic CdR levels as other parameters when evaluating responses in certain clinical trials involving the use of TdR alone or in combination.

[6]Booklet No. 10: L1210 leukemia: response to ara-C. Southern Research Institute, Birmingham, AL, Dec 1976.

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# Levodopa and Dopamine Analogs: Dihydroxy and Trihydroxybenzylamines as Novel Quinol Antitumor Agents in Experimental Leukemia In Vivo [1,2]

Michael M. Wick [3,*]

## SUMMARY

**Levodopa and dopamine demonstrate significant antitumor activity in several experimental systems. We have prepared the nonneurotoxic dihydroxybenzylamine (DHBA) analogs 2,3-DHBA, 3,4-DHBA, and 2,5-DHBA and the trihydroxy derivatives 2,3,4- and 3,4,5-trihydroxybenzylamine. These analogs demonstrated significant and reproducible antitumor activity in the ip P388 and L1210 lymphocytic leukemias. This activity was markedly increased when the drugs were given by multiple injections three times daily for 4 days. 3,4-DHBA and 2,3-DHBA resulted in 30% and 20% long-term survivors, respectively. There was a selective inhibition of thymidine incorporation and a relatively lesser effect on uridine and leucine incorporations. Inhibitory concentrations were between 0.1 and 1.0 mM. The trihydroxy derivatives were more potent, with inhibitory concentrations between 0.01 and 1.0 mM. Furthermore, the trihydroxy derivatives were also able to inhibit the incorporation of uridine and leucine as well as thymidine. The *para* derivative, 2,5-DHBA, although a potent inhibitor in vitro, was completely inactive in vivo. When L1210 and P388 tumor-bearing animals were given radioactively labeled thymidine in vivo following the administration of drugs, a selective inhibition of thymidine incorporation by tumor cells was observed, with essentially no effect on gut or bone marrow. Doses > 200 mg/kg completely suppressed the incorporation of radioactively labeled thymidine by tumor cells 1 hour after administration of drug. A similar dose response was observed in the more slowly growing P388 leukemia, suggesting that the antitumor effect did not strongly correlate with growth rate of the tumor. Since levodopa and dopamine are currently being evaluated in patients with metastatic melanoma, the availability of analogs with enhanced antitumor activity and broader antitumor spectrum are of interest.**

[Cancer Treat Rep 65:861–867, 1981]

Levodopa and dopamine are effective antitumor agents in several experimental tumor systems including B16 melanoma, C1300 neuroblastoma, and L1210 and P388 lymphocytic leukemias (1–5). Furthermore, levodopa and dopamine are currently undergoing clinical evaluation for the treatment of human metastatic melanoma with promising initial results. The biochemical effects of these drugs on tumor cells are characterized by a rapid and selective inhibition of thymidine incorporation, arrest of cell cycle traverse at the $G_1$/S interface, and inhibition of the incorporation of radioactively labeled thymidine triphosphate by permeabilized human melanoma cells. These observations are consistent with the inhibition of cell growth occurring as a result of inhibition of DNA synthesis. Furthermore,

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[3]Division of Medical Oncology and Pharmacology, Sidney Farber Cancer Institute, and Department of Dermatology, Harvard Medical School, Boston, MA.

**Reprint requests to:* Michael M. Wick, MD, Charles A. Dana Cancer Center, Sidney Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

the potent inhibitory effects of the oxidation products of these quinols on isolated mammalian DNA polymerase α suggests that this enzyme is at least one site of action (6). In this system, however, the reduced quinols themselves demonstrate no inhibitory activity, requiring the presence of an oxidative enzyme such as tyrosinase or chemically preformed quinones. Although cytotoxicity is quantitatively greater in oxidase-containing cells such as melanoma cells, other tumors, especially those with rapid growth rates such as the L1210 leukemia, are also sensitive to the antitumor effect. It is possible that alternative mechanisms of action are operative in these nonmelanocytic cells.

Preliminary structure-activity studies suggested that the cytotoxic properties of these molecules lay in the *ortho*-dihydroxybenzene portion of the aromatic ring, while toxicity was primarily related to their catecholamine activity. An increase in antitumor activity through the diminishing of the prominent neuroagonist activity of these drugs was achieved by the preparation of a derivative, 3,4-dihydroxybenzylamine, an observation that has been independently confirmed (7). In both the L1210 lymphocytic leukemia as well as the B16 melanoma, this derivative demonstrated enhanced antitumor effect (8,9). In order to define the structural parameters that are responsible for activity in this system as well as to maximize the antitumor activity of these agents, we undertook the synthesis and evaluation of a series of dihydroxybenzylamines (DHBA) and trihydroxybenzylamines (THBA).

## MATERIALS AND METHODS

*Drugs.*—Each analog was prepared synthetically and the details of the chemical synthesis of the DHBA and THBA will be reported elsewhere. All chemicals used in this study were reagent grade and were obtained from the Sigma Chemical Co (St. Louis, MO). Radioactively labeled [methyl-$^3$H]thymidine (specific activity, 40–60 curies/mmol), [5-$^3$H]uridine (specific activity, 40–60 curies/mmol), and L-[4,5-$^3$H]leucine (specific activity, 20 curies/mmol) were from the New England Nuclear Corp (Boston, MA).

*In vivo antitumor assays.*—$BDF_1$ ([C57BL/6 × DBA/2]$F_1$) male mice 6–8 weeks of age were obtained from The Jackson Laboratory (Bar Harbor, ME). Assay procedures were identical to those of the standard National Cancer Institute Protocols (10). All drugs were administered ip in 0.9% sodium chloride. Antitumor activity was determined by measuring the increase in lifespan in mice given $1 \times 10^5$ L1210 or $1 \times 10^6$ P388 lymphocytic leukemia cells ip on Day 0. The treatment was begun on the day after tumor inoculation. The percent increase in lifespan was calculated according to the formula: % T/C = (T/C) × 100, where T and C are the median survival time in days of the treatment and the control groups, respectively. Significance tests were performed by analysis of variance. Toxic deaths were defined as animals dying before controls, and only doses that did not cause toxicity were subjected to analysis. The % T/C was calculated based on dying animals only, with survivors (> 60 days) reported separately.

*In vitro assays.*—The maintenance and origin of the L1210 lymphocytic leukemia and CCRF-CEM cells have been described (11). The cells were maintained in suspension culture in Eagle's minimum essential medium containing 10% fetal bovine serum, 100 μg/ml of streptomycin, and 100 units/ml of penicillin in a 5% $CO_2$ humidified air incubator at 37°C. The medium was also supplemented with 0.05 mM 2-mercaptoethanol. The techniques for growth inhibition and for radiolabeled precursor incorporation studies have been described (8). A total of $5 \times 10^5$ cells/ml were exposed to 1 microcurie/ml of radioactively labeled precursor and the appropriate concentration of drugs for 60 minutes. The cells were filtered onto a Whatman GF/C glass filter and washed once with 10% trichloroacetic acid and three times with a 0.9% sodium chloride. The filters were then digested in Protosol (New England Nuclear Corp), placed in scintillation vials containing Aquasol (New England Nuclear Corp), and counted in a Beckman LS-335 scintillation counter. All determinations were in triplicate, with values expressed as the mean ± standard error of the mean for three to five experiments or reported as a percent of the control.

*In vivo assays.*—The procedures for assessing incorporation of radioactively labeled thymidine into murine tumor, gastrointestinal mucosa, or bone marrow in vivo have been reported (12). $BDF_1$ mice were used on the 4th day following the inoculation of $10^5$ L1210 or $10^6$ P388 lymphocytic leukemia cells. At the indicated times following the administration of drug, the mice were given 50 microcuries of tritiated thymidine by ip injection. One hour later, the animals were killed by cervical dislocation. The femurs were isolated and irrigated with normal saline to remove the nucleated bone marrow cells. The tumor cells were harvested by ip lavage. A 2-cm section of proximal duodenum was removed and its mucosa was separated. DNA was extracted (13) and determined colorimetrically (14), and an aliquot of the extract was subjected to liquid scintillation counting in Aquasol. Each determination was performed in triplicate. The values are expressed as counts per minute per microgram of DNA and are compared to untreated controls.

TABLE 1.—Effect of daily treatment with DHBA and THBA on L1210 and P388 leukemias in vivo*

| Drug | Dose (mg/kg) | Lifespan of mice given— L1210 Range (days) | L1210 Median (days) | L1210 % T/C | P388 Range (days) | P388 Median (days) | P388 % T/C |
|---|---|---|---|---|---|---|---|
| 3,4-DHBA | 400 | 10–15 | 13 | 144 | 13–16 | 14 | 141 |
| | 500 | 11–16 | 14 | 155 | 15–23 | 17 | 150 |
| | 600 | 11–17 | 14 | 155 | 16–24 | 18 | 165 |
| 2,3-DHBA | 400 | 13–15 | 14 | 155 | 16–19 | 14 | 145 |
| | 500 | 13–15 | 14 | 155 | 16–25 | 15 | 154 |
| | 600 | 13–14 | 14 | 155 | 16–25 | 16 | 163 |
| 2,5-DHBA | 100–600 | | | Inactive | | | |
| 2,4-DHBA | 100–600 | | | Inactive | | | |
| 2,3,4-THBA | 100 | 11–13 | 12 | 133 | 16–19 | 12 | 120 |
| | 200 | 12–14 | 12 | 133 | 16–20 | 16 | 145 |
| | 250 | 12–15 | 13 | 144 | 16–20 | 16 | 145 |
| 3,4,5-THBA | 100 | 12–14 | 12 | 133 | 16–19 | 12 | 120 |
| | 200 | 12–14 | 12 | 133 | 15–22 | 17 | 154 |
| | 250 | 11–13 | 13 | 144 | 15–21 | 17 | 154 |

*Median values for controls were 8.5–9.0 days for L1210 (range, 8–11) and 10–11.5 days for P388 (range, 10–14). There were 10 mice/group and each experiment was repeated 3 times with similar results.

TABLE 2.—Enhancement of antitumor response to DHBA in L1210 and P388 leukemias by more frequent administration*

| Drug | Dose (mg/kg) | Lifespan of mice given— L1210 Range (days) | L1210 Median (days) | L1210 % T/C | P388 Range (days) | P388 Median (days) | P388 % T/C |
|---|---|---|---|---|---|---|---|
| 3,4-DHBA | 100 | 11–17 | 13 | 163 | 16–17 | 17 | 154 |
| | 200 | 11–17 | 15 | 187 | 16–19 | 18 | 163 |
| | 300 | 13–22 | 16 | 191 (10) | 19–24 | 22 | 200 |
| | 400 | 13–22 | 22 | 225 (40) | 16–24 | 24 | 208 (10) |
| 2,3-DHBA | 100 | 13–21 | 13 | 147 | 16–17 | 17 | 145 |
| | 150 | 19–21 | 21 | 233 (10) | 16–17 | 17 | 145 |
| | 200 | 15–24 | 20 | 188 (40) | 15–17 | 17 | 145 (10) |
| | 250 | 19–20 | 22 | 222 (40) | 16–25 | 18 | 163 (10) |

*Drugs were administered 3 times daily for 4 days beginning 1 day after tumor inoculation. Median values for controls were 8.5–9.0 days for L1210 (range, 8–11) and 10–11.5 days for P388 (range, 10–14). There were 10 mice/group and each experiment was repeated 3 times with similar results. Values in parentheses = % survivors at 60 days (defined as "cures").

## RESULTS

Tables 1–3 summarize the antitumor data against L1210 and P388 leukemias in vivo for each drug. When given daily for 7 days beginning on the day after tumor inoculation (table 1), reproducible antitumor activity was observed with the DHBA and THBA derivatives that possess an *o*-dihydroxybenzene feature in their structure. Significant antitumor activity was manifest against both the L1210 and P388 lymphocytic leukemia models. However, the *p*-benzoquinol derivative, 2,5-DHBA, representing the only compound that is potentially a *para*-benzoquinone, showed no antitumor activity. Similarly, the 2,4-DHBA, a *meta* derivative, which is unable to undergo oxidation to a quinone, did not show activity.

Tables 2 and 3 describe the effects of treatment using a more frequent schedule of administration. The drugs were given three times daily for 4 days beginning on the day after tumor inoculation. As reported previously, when 3,4-DHBA is adminis-

TABLE 3.—Effect of more frequent administration of THBA on antitumor response of L1210 and P388 leukemias*

| Drug | Dose (mg/kg) | Lifespan of mice given— L1210 Range (days) | L1210 Median (days) | L1210 % T/C | P388 Range (days) | P388 Median (days) | P388 % T/C |
|---|---|---|---|---|---|---|---|
| 2,3,4-THBA | 50 | 11–17 | 14 | 155 | 17–19 | 17 | 147 |
| | 75 | 11–14 | 14 | 155 | 18–20 | 19 | 165 (10) |
| | 100 | 11–18 | 15 | 166 | 19–21 | 19 | 165 (10) |
| 3,4,5-THBA | 50 | 11–14 | 11 | 155 | 17–19 | 15 | 150 |
| | 75 | 11–14 | 11 | 155 | 19–21 | 20 | 175 |
| | 100 | 11–17 | 14 | 155 (20) | 19–24 | 20 | 175 |

*Drugs were administered 3 times daily for 4 days beginning on the day after tumor inoculation. Median values for controls were 8.5–9.0 for L1210 (range, 8–11) and 10–11.5 for P388 (range, 10–14). There were 10 mice/group and each experiment was repeated 3 times with similar results. Values in parentheses = % survivors at 60 days (defined as "cures").

TABLE 4.—Effect of various concentrations of DHBA and THBA on the incorporation of thymidine, uridine, and leucine by L1210* cells in vitro

| | % inhibition of — Thymidine 0.01 mM | Thymidine 0.1 mM | Thymidine 1.0 mM | Uridine 0.01 mM | Uridine 0.1 mM | Uridine 1.0 mM | Leucine 0.01 mM | Leucine 0.1 mM | Leucine 1.0 mM |
|---|---|---|---|---|---|---|---|---|---|
| 3,4-DHBA | 0† | 35 | 90 | 0 | 0 | 13 | 10 | 15 | 17 |
| 2,3-DHBA | 14 | 73 | 97 | 0 | 0 | 16 | 21 | 31 | 38 |
| 2,3,4-THBA | 27 | 33 | 97 | 0 | 28 | 63 | 0 | 10 | 37 |
| 3,4,5-THBA | 30 | 56 | 94 | 0 | 25 | 48 | 19 | 40 | 52 |
| 2,5-DHBA | 0 | 80 | 99 | 4 | 60 | 96 | 22 | 51 | 67 |
| 2,4-DHBA | 0 | 0 | 20 | | 0 | | | 0 | |

*Similar patterns were observed with CEM human leukemia cells.

†Values = % inhibition as compared to controls for 5 separate determinations. Control incorporations were 4320 cpm/$10^4$ cells for thymidine, 6734 cpm/$10^4$ cells for uridine, and 860 cpm/$10^4$ cells for leucine.

tered with decreased dose intervals, a significant improvement in antitumor activity is observed in both the L1210 and P388 leukemias. The improvement is especially apparent in the case of the dihydroxy derivatives 3,4-DHBA and 2,3-DHBA. With these drugs, significant numbers (30%) of long-term survivors begin to appear in both the L1210 and P388 systems. A modest improvement in the antitumor effect was seen with 2,3,4-THBA and 3,4,5-THBA, with an occasional cure observed. In contrast to these results, no significant improvement was observed with 2,5-DHBA, indicating that this derivative does not benefit from being administered on a more frequent schedule.

A characteristic feature of the *o*-quinols is their ability to selectively inhibit thymidine incorporation with relatively lesser effects on uridine and leucine incorporation. Table 4 summarizes the comparative effects on macromolecule precursor incorporation. The *o*-quinols 2,3-DHBA and 3,4-DHBA caused a relatively selective inhibition of thymidine incorporation in contrast to the trihydroxy derivatives 2,3,4-THBA and 3,4,5-THBA, which caused significant inhibition of leucine and uridine incorporation also (at the 1 mM concentration). These results suggest that the mechanism of action of these two types of derivatives might not be identical. 2,4-DHBA demonstrated no effect on macromolecule incorporation up to a concentration of 1 mM, while the 2,5-DHBA was a very potent inhibitor in vitro.

Since the compounds differ substantially in their antitumor activity, we wished to determine what portion of this difference could be attributed to differences in cytotoxicity. Table 5 depicts the in vitro inhibitory effects of the various drugs on L1210 lymphocytic leukemia and CEM human leukemia cells in vitro following 48 hours of continuous exposure. The most potent compounds were 2,3,4-THBA, 3,4,5-THBA, and 2,5-DHBA, while 2,4-DHBA, which is unable to undergo oxidation to a quinone, was noncytotoxic as anticipated. For 2,5-DHBA, the results are in marked contrast to the in vivo system, where

TABLE 5.—Effect of DHBA and THBA on growth of L1210 leukemia and CCRF-CEM cells in vitro

| Drug | ID50 concentration (mM) for — | |
|---|---|---|
| | L1210 cells | CEM cells |
| 3,4-DHBA | 0.170* | 0.150 |
| 2,3-DHBA | 0.140 | 0.120 |
| 2,5-DHBA | 0.034 | 0.021 |
| 2,4-DHBA | > 1.000 | > 1.000 |
| 2,3,4-THBA | 0.021 | 0.019 |
| 3,4,5-THBA | 0.035 | 0.028 |

*Values = concentration causing a 50% reduction in growth of exponentially growing cells following a 48-hr exposure. 5 dose levels were tested with each point in triplicate. ID50 values were determined by computer fit of data.

this drug is completely inactive. Interestingly, the more selective dihydroxy derivatives appear to be less cytotoxic.

Table 6 summarizes the effects of DHBA and THBA on thymidine incorporation in tumor-bearing mice, comparing the effects on tumor to those on other rapidly proliferating tissues (bone marrow and gastrointestinal mucosa). 2,3-DHBA and 3,4-DHBA demonstrated similar selectivity and potency toward L1210 cells. As measured 1 hour after drug exposure, a selective inhibition of incorporation into tumor occurred, with substantially less effect on bone marrow and gastrointestinal mucosa. Selectivity of action is manifest in the suppression of incorporation of radioactively labeled thymidine into tumor cells at doses causing little or no inhibition on thymidine incorporation by bone marrow or mucosal cells. At the highest doses tested, modest suppression of incorporation by bone marrow and gastrointestinal mucosa was also observed.

Using the more potent trihydroxy derivatives 2,3,4-THBA and 3,4,5-THBA, there was complete suppression of tumor incorporation but also significant inhibition of thymidine incorporation into marrow and gut. Therefore, it appears that the diminished antitumor efficacy of these drugs is related, in part, to decreased selectivity, and the enhanced cytotoxicity observed in vivo also affects normal proliferating tissues.

A similar situation exists with the *para* derivative, 2,5-DHBA. Although in short-term experiments this compound was effective in inhibiting the incorporation of thymidine by tumor cells, there was significant inhibition of gut and marrow, perhaps accounting for the absence of antitumor action.

Table 6 also shows the effects of drugs at the same doses on the slower growing P388 leukemia. Although this tumor grows significantly more slowly than L1210, comparable inhibition of thymidine incorporation occurred, indicating that antitumor effect is not entirely a function of the growth rate. At doses > 200 mg/kg, there was essentially complete suppression of thymidine incorporation by tumor cells with each of the drugs tested except 2,5-DHBA. The effects on bone marrow and gastrointestinal mucosa were similar to those observed in L1210-bearing mice.

The examination of effects on thymidine incor-

TABLE 6.—Effect of DHBA and THBA on incorporation of radioactively labeled thymidine in L1210 and P388 tumor-bearing mice

| Drug | Dose (mg/kg) | % of control incorporation | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tumor | | Bone marrow | | Gastrointestinal mucosa | |
| | | L1210 | P388 | L1210 | P388 | L1210 | P388 |
| 3,4-DHBA | 200 | 1 | 5 | 60 | 60 | 170 | 110 |
| | 400 | 1 | 2 | 70 | 80 | 140 | 100 |
| | 600 | 1 | 1 | 60 | 70 | 100 | 100 |
| 2,3-DHBA | 200 | 32 | 20 | 90 | 80 | 140 | 110 |
| | 400 | 5 | 12 | 85 | 80 | 135 | 100 |
| | 600 | 1 | 8 | 60 | 70 | 48 | 70 |
| 2,3,4-THBA | 200 | 2 | 14 | 50 | 15 | 140 | 120 |
| | 400 | 1 | 11 | 35 | 35 | 52 | 50 |
| | 600 | 1 | 7 | 10 | 27 | 20 | 24 |
| 3,4,5-THBA | 200 | 75 | 15 | 100 | 100 | 135 | 110 |
| | 400 | 25 | 12 | 45 | 50 | 65 | 75 |
| | 600 | 1 | 5 | 40 | 40 | 40 | 80 |
| 2,5-DHBA | 200 | 115 | 100 | 130 | 110 | 110 | 115 |
| | 400 | 65 | 90 | 150 | 94 | 105 | 120 |
| | 600 | 45 | 70 | 85 | 75 | 60 | 80 |

*Values = % of control for 3 determinations. Tumor cell incorporation was 600 cpm/μg DNA. The mice were used on Day 4 following implantation of $10^6$ P388 or $10^5$ L1210 cells and were processed as before.

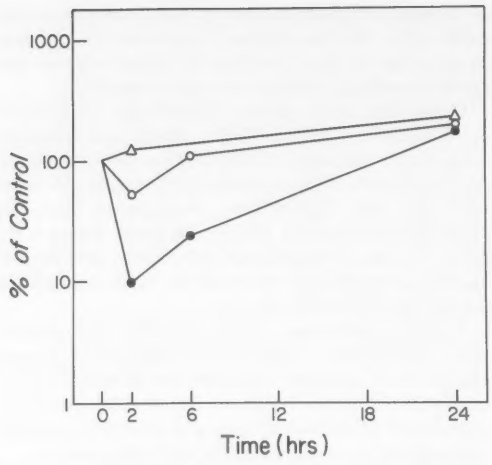


FIGURE 1.—Recovery of thymidine incorporation by L1210 cells (●), bone marrow (○), and gastrointestinal mucosa (Δ) following treatment with 3,4-DHBA. At the indicated times following treatment with drug, mice were killed and processed as described. Control values were 18,000 cpm/μg DNA for L1210, 475 cpm/μg DNA for bone marrow, and 650 cpm/μg DNA for gastrointestinal mucosa.

poration in vivo provides a means for estimating the duration of effect of a single dose. Figure 1 shows recovery in vivo following administration of 3,4-DHBA to L1210-bearing mice. The effects were greatest approximately 1 hour after exposure, while at 6 hours there was major recovery that was complete at 24 hours. These results correlate well with effect of administration schedule on antitumor response since more frequent administration caused enhancement of antitumor activity. The administration of drug at 3–4-hour intervals effectively inhibited tumor repopulation.

## DISCUSSION

The *ortho*-quinols reported here represent a new class of antitumor agents. Since the original report of the antitumor activity of levodopa, which also exhibits antitumor activity against the nonmelanocytic leukemia tumors P388 and L1210, it was apparent that the spectrum of activity of these agents was not limited to tyrosinase-containing cells. This paper extends these results and demonstrates that with alteration of schedule, substantial activity in the leukemia models can be obtained. Of particular interest is the derivative 3,4-DHBA, which is also active (% T/C = +170) in the B16 melanoma model (9). The basis for schedule dependence of antitumor agents has been well-summarized and heretofore was best illustrated in the case of cytarabine (15,16). Using the more frequent schedules causes the appearance of long-term survivors, indicating that tumor kills in excess of five logs are obtainable. It might be anticipated that further improvement of efficacy might be achieved by continuous exposure of tumor cells to drug, for example, by continuous infusion.

The inhibition of DNA synthesis appears to be central to the mechanism of action of these agents. In tyrosinase-containing cells, such as melanoma cells, DNA polymerase can be inhibited by the oxidation of products of the quinols. The precise mechanism of action in leukemia cells, however, is less clear. It is possible that other oxidases are present and cause oxidation of the quinones, or alternatively, the quinols themselves can inhibit other enzymes such as ribonucleotide diphosphate reductase. Either mechanism would account for the biochemical changes observed.

Interestingly, the trihydroxy derivatives appear to be both more cytotoxic as well as less selective in their biochemical effects. These results indicate that the additional hydroxyl group has a marked influence on the biologic properties of the drug, possibly by lowering its redox potential. This possibility is further supported by the increased cytotoxicity of the *para*-dihydroxy isomer. The lack of in vivo activity might alternatively be attributed to a metabolic or pharmacokinetic disadvantage.

Finally, the preliminary results of our clinical evaluation of levodopa and dopamine in humans with tumors are encouraging. With the availability of these nonneurotoxic analogs, the potential exists for the enhancement of activity as well as extension to other tumor types (17,18).

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# Melphalan, Vincristine, and 5-FU in Advanced Colonic Carcinoma: Results of a Pilot Study[1]

Brief Reports and Preliminary Communications

Julia Ashenhurst, Jacob D. Bitran,* Erwin Robin, Richard K. Desser, Charles Shapiro, Mark F. Kozloff, and Arthur A. Billings[2]

## SUMMARY

**The results of treatment with melphalan, vincristine, and 5-FU (MVF) in 32 patients with advanced colonic carcinoma are reported. The objective response rate among 31 patients with measurable disease was 16% (five patients responded). The projected survival for all 32 patients was 277 days. Patients with objective response to MVF had a projected survival of 314 days, significantly longer than the survival of patients with no response to therapy, 84 days ($P = 0.01$). While toxic effects were mild with MVF, this regimen appears to have no greater activity in advanced colonic carcinoma than therapy with 5-FU alone or in combination with nitrosoureas.**

[Cancer Treat Rep 65:869–872, 1981]

The optimal treatment of colorectal carcinoma has been the subject of an intense research effort for the past two decades. Yet, despite this research in the treatment of advanced colonic carcinoma, the best and most efficacious drug or combination of drugs is still unknown. 5-FU, cyclophosphamide, methyl-CCNU, mitomycin, and triazinate are active agents in patients with advanced colonic carcinoma (1,2). Combinations of methyl-CCNU and 5-FU, with or without vincristine, have yielded variable response rates (4%–50%) in patients with advanced colon carcinoma (1); however, despite high response rates as reported by some groups, there is no conclusive evidence that survival is prolonged in patients with advanced colonic carcinoma receiving the two- or three-drug combination as compared to 5-FU alone (1,3).

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[2]Division of Hematology/Oncology, Department of Medicine, Michael Reese Hospital and Medical Center, Pritzker School of Medicine, Chicago, IL.

*Reprint requests to: Dr. Jacob D. Bitran, Division of Hematology/Oncology, Department of Medicine, Michael Reese Hospital and Medical Center, 29th St and Ellis Ave, Chicago, IL 60616.

While many groups have utilized 5-FU and the nitrosoureas for colorectal carcinoma, few investigators have employed other drugs that may be active, such as cyclophosphamide, methotrexate, and melphalan (4–7). Bullen et al (4) have reported that melphalan is equal in its activity (12.5% objective response rate) to 5-FU in advanced gastrointestinal cancer, including colorectal carcinoma. Furthermore, melphalan produced less nausea, vomiting, diarrhea, and myelosuppression than did 5-FU (4).

In 1979, we initiated a pilot program using melphalan, vincristine, and 5-FU (MVF) in the treatment of patients with advanced colonic cancer. The objectives of this study were to ascertain whether this regimen was efficacious and to determine if serial measurements of carcinoembryonic antigen (CEA) were helpful in predicting response to treatment. We report here the results of therapy with MVF in patients with advanced colonic carcinoma.

## MATERIALS AND METHODS

From January 1, 1979, to April 30, 1980, 32 patients with advanced colon carcinoma (16 males and 16 females; median age, 64 years [range, 47–79]) were entered in a pilot chemotherapy study using MVF. Of the 32 patients, 21 were previously untreated, while the remaining 11 had received either radiotherapy (six patients), 5-FU alone (four), or 5-FU and methyl-CCNU (one). Thirty-one of the 32 patients had measurable disease: hepatomegaly extending below the costal margin (20 patients), abdominal masses (seven), palpable adenopathy (two), and pulmonary nodules (two). A liver-spleen scan showed that one patient had metastases and no hepatomegaly. This patient is the only patient in this series with evaluable disease.

The median Karnofsky performance status for the entire group of 32 patients was 70, with a range of 30–100 (table 1).

The chemotherapy program consisted of 0.2 mg/kg/day of melphalan on Days 1–5; 1.4 mg/m$^2$ of vincristine iv (maximum, 2 mg) on Days 1, 8, and 15;

TABLE 1.—Response rate and median survival as stratified by Karnofsky performance status

| Performance status | No. of patients with— | | Median survival (days) | |
|---|---|---|---|---|
| | Measurable disease | Objective response (rate) | | |
| 100–80 | 14 | 3 (21%) | 330 | $P = 0.01$ |
| 70–60 | 10 | 1 (10%) | 121 | $P = 0.08$ |
| < 60 | 7 | 1 (14%) | 69 | |

and 600 mg/m² of 5-FU iv on Days 1, 8, 15, 22, and 28. The program was repeated every 42 days.

Prior to the start of MVF treatment and before each chemotherapy cycle, patients had a complete physical examination, chest roentgenogram, urinalysis, SMA-12, and determination of plasma CEA levels. Cbcs were performed weekly while patients received chemotherapy. In patients with hepatic involvement, the liver scan was repeated after the first and second courses of therapy and every 4 months thereafter. The plasma CEA values were determined by direct radioimmunoassay using the method of Thomas et al (8). A CEA level > 5 ng/ml was considered abnormal. Fluctuations < 25% in the CEA level were not considered to be significant changes.

Complete and partial responses were defined by the usual criteria. A reduction in the sum products of all measurable lesions by < 50% or no change without the appearance of new lesions for at least 2 months was considered stable disease. Progressive disease was designated as no response.

A life-table analysis was performed and the actuarial survival curves were compared by the Gehan modification of the generalized Wilcoxon test (9). Proportions among independent samples were compared by the use of Fisher's exact test.

## RESULTS

Five of the 31 patients with measurable disease responded, yielding an objective response rate of 16% (95% confidence limits, 3%–29%). The objective response rate among 21 previously untreated patients was 19% (four responded). Only one of ten (10%) previously treated patients had an objective response. All responses were partial and occurred within the first course (42 days) of MVF. No additional responses occurred after the initial course of therapy. The median duration of response was 243 days. Thirteen of the 32 patients are still alive.

The highest objective response rate was observed in the 14 patients with a performance status of 80–100 (table 1). As the performance status decreased, so did the response rate and median survival time.

The median survival was 277 days for all 32 patients, 314 days for patients with an objective response to MVF, and 84 days for patients with no response to MVF ($P = 0.01$ by Wilcoxon test). The median survival for the nine patients with stable disease has not been reached and is estimated to be > 150 days.

The hematologic toxic effects from MVF were relatively mild. The nadir wbc count (mean, 4500/mm³ with 1510 granulocytes/mm³) and platelet count (mean, 158,000/mm³) usually occurred on Days 14 and 21, respectively. Only one patient had a granulocyte count < 1000/mm³, and three patients had platelet counts < 100,000/mm³. Other toxic effects included mild nausea and vomiting with melphalan in five patients (15%), paresthesias secondary to vincristine in seven (21%), and oral ulceration as a result of 5-FU in two (4%).

Six patients' characteristics were examined to determine their relationship to response and survival. These included age, sex, performance status, time from initial diagnosis to stage D, site of metastasis, and percent of MVF dose administered. Only performance status (table 1) and the time to stage D disease were significant variables. Patients who presented with stage D colonic carcinoma or developed distant metastases within 6 months of "curative surgery" had a longer projected median survival (310 days) than those who developed distant metastases > 12 months after curative surgery (120 days) ($P = 0.005$). This difference in survival can be accounted for by the higher objective response rate (38%; five responses) among 13 patients who presented with or quickly developed stage D disease as compared to the lack of objective responses in the 18 patients who developed stage D disease at ≥ 12 months ($P = 0.01$ by Fisher's exact test). Performance status and site of metastasis were similar in these two subgroups, so the apparent difference could not be explained by a delay in diagnosis of stage D, or "lead time."

An objective of this pilot study was to determine the predictive value of plasma CEA determinations relative to survival. All 32 patients had increased pretreatment CEA levels (> 5 ng/ml) and had serial CEA values determined every 6 weeks. Twelve patients had significant decreases in CEA level (> 75% reduction of the pretreatment value), six had no change, and 13 had increasing levels. When survival was plotted on the basis of a decreasing, unchanged, or increasing CEA level, patients with a decreasing CEA level had a significantly longer median survival (> 330 days) than those with increasing or static CEA levels (115 days) ($P = 0.01$).

## DISCUSSION

The reason for using a melphalan-based regimen in patients with advanced colonic carcinoma was to exploit any potential synergism between melphalan and 5-FU. Melphalan was chosen because of its reported activity (2,4) and relatively mild gastrointestinal and hematologic toxicity. While toxicity with MVF was mild, the response rate achieved with this regimen and survival of treated patients were no better than reported results with 5-FU alone or in combination with methyl-CCNU (1,3,10–14). Response to MVF occurred in few patients (16%), and while response occurred rapidly, its duration was short (243 days). In our analysis of patient characteristics, performance status was an important prognostic variable relative to response and survival. This observation has been reported by others (13). The time interval to stage D colonic cancer was also important. While exact time intervals are difficult to determine, many patients in our series had been followed closely after an initial "curative resection" for colonic carcinoma. It is interesting that patients with slowly growing tumors, as evidenced by a > 12-month interval to stage D, had a more limited survival than patients with more "biologically aggressive" tumors (metastases at laparotomy or within 6 months of curative resection). We believe this paradox may be explained by the strikingly higher response rate (35%) among the 13 patients with biologically aggressive colonic neoplasms as compared to the zero response rate in patients with more indolent colonic carcinoma. Since age, sex, and performance status were matched in these groups, we believe this to be the only plausible explanation.

Our results as to the predictive value of serial CEA determinations confirm the reports of Al-Sarraf et al (15) and Shani et al (16) in that a correlation existed between a decrease in the pretherapy CEA level and clinical regression, and a statistically superior survival was found among patients with a decreasing CEA titer as compared to patients with stable or increasing titers. In patients with nonmeasurable and nonevaluable lesions (bone metastases), where no clear-cut endpoint exists in determining response to therapy, a decreasing plasma CEA level may be interpreted as indirect evidence of response.

It is not clear from our trial with MVF as to whether melphalan alone or 5-FU alone would have achieved equally mediocre results. Our suspicions that 5-FU alone may be of equal efficacy have been confirmed by recent reports (5,7). Thus, the addition of an alkylator in the treatment of colonic cancer yields little therapeutic benefit (5,7). Clearly, phase II trials with new drugs in patients with colonic carcinoma are warranted.

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# Initial Clinical Studies of Vindesine[1,2,3]



Manuel Valdivieso,* Stephen Richman, Andrew M. Burgess, Gerald P. Bodey, and Emil J Freireich[4]


## SUMMARY

**Vindesine, a newer vinca alkaloid derivative, underwent a phase I clinical evaluation in 68 humans with advanced, refractory malignancies. Patients received single total doses ranging from 2 to 12.5 mg, repeated every 1–2 weeks. Dose-limiting myelosuppressive, gastrointestinal, and neurologic toxic effects were observed at higher doses. They consisted predominantly of neutropenia, constipation leading, on occasion, to paralytic ileus, and peripheral neuropathy. Total doses of 7.5–10 mg (equivalent to 4–5 mg/m²), repeated every 2 weeks, were well-tolerated. There were two partial remissions in patients with acute leukemia and prior vincristine therapy, two minor responses in patients with renal cell carcinoma, one minor response in a patient with squamous cell carcinoma of the esophagus, and one minor response in a patient with squamous cell carcinoma of the lung. Vindesine is well-tolerated by man, although it shares some of the toxic manifestations of vinblastine and vincristine. Its efficacy in some patients who were no longer responsive to vincristine therapy suggests a lack of clinical cross-resistance between these compounds.**


[Cancer Treat Rep 65:873–875, 1981]

The vinca alkaloids are a potent group of antitumor agents with a wide spectrum of activity in human cancers. However, the usefulness of the vinca alkaloids remains limited because of the significant side effects associated with their administration. Myelosuppression and gastrointestinal side effects, including paralytic ileus, are major drawbacks of vinblastine therapy; progressive peripheral neuropathy, which at times becomes incapacitating, is the major toxic manifestation of vincristine therapy.

Vindesine is a newer member of the vinca alkaloid family which was developed from vinblastine. In animal tumor systems, the activity of vindesine is broader than that of the parent compound, and is similar to that of vincristine. In addition, vindesine was found to be devoid of neurotoxicity (1,2). Because of the potential clinical implications of this compound, a phase I clinical study was undertaken.

## PATIENTS AND METHODS

Adults with advanced malignant disease refractory to standard chemotherapeutic agents were entered in this study. Included were patients whose prior therapy contained vinca alkaloid derivatives. Signed informed consent forms were obtained according to institutional policies. Vindesine was obtained from Eli Lilly and Co, Indianapolis, IN.

Vindesine was administered as single intermittent iv infusions; the initial total dose was 2 mg. During subsequent courses of treatment, the dose of vindesine was progressively increased by 50%–100%, although other dose modifications were done according to patient tolerance. Each dose of vindesine was dissolved in 100 ml of 5% dextrose solution over a 15- to 30-minute period. Courses of therapy were repeated every 1–2 weeks, depending on the patient's complete recovery from toxic effects. Patients were closely monitored, and all side effects noticed during drug administration, as well as those developed between courses of treatment, were recorded.

Cbcs were performed twice weekly; urinalysis and SMA-100 determinations were performed once weekly. Appropriate radiologic examinations, as well as radioisotope-scanning procedures, were usually

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[3]Presented in part at the 67th Annual Meeting of the American Association for Cancer Research, Toronto, Canada, May 4–8, 1976.

[4]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Dr. Manuel Valdivieso, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Nonhematologic toxic effects of vindesine

| | Toxicity of vindesine at — | | |
|---|---|---|---|
| | 2–6 mg | 7.5 mg | 10–12.5 mg |
| % patients with toxic effects | 58 | 70 | 97 |
| % courses with toxic effects | 39 | 43 | 70 |
| Constipation | 8 | 16 | 30 |
| Paralytic ileus | 2 | 4 | 2 |
| Nausea, vomiting, and diarrhea | 12 | 15 | 27 |
| Chills and fever | 2 | 4 | 21 |
| Paresthesias | 6 | 12 | 19 |
| Weakness | 4 | 5 | 19 |
| Alopecia | 2 | 7 | 14 |
| Phlebitis and cellulitis | 14 | 16 | 13 |
| Other* | — | 6 | 8 |

*5 episodes of arthralgias, 2 of confusion and lethargy, 2 of heartburn, and 1 each of anorexia, jaw pain, and hallucination.

performed after every two to three courses of treatment. Tumor measurements were recorded before each treatment. The criteria for tumor response were standard.

## RESULTS

Sixty-eight adults (45 males and 23 females) were entered in this study; their median age was 49 years (range, 16–77). Patient malignancies included: acute leukemia (19 patients); renal cell carcinoma (13); colorectal carcinoma (eight), malignant melanoma (eight); lung cancer (five); breast cancer (three); head and neck cancer (three); sarcoma (three); lymphoma (two); and esophageal cancer, undifferentiated of unknown primary, adenocarcinoma of unknown primary, and gallbladder carcinoma (one each).

The initial total vindesine dose was 2 mg and it was increased to 4, 5–6, 7.5, 9–10, and 12.5 mg, successively. Of 244 treatment courses, most were given at doses of 7.5 mg (117 courses) and 9–10 mg (61 courses).

The hematologic toxicity of vindesine consisted primarily of neutropenia, with little effect on platelet and rbc counts. At doses < 7.5 mg, 80%–90% of all treatments were not associated with significant myelosuppression. In fact, three patients who received doses of 2 and 4 mg and who had no myelosuppression developed a rising platelet count (double their pretreatment values) during therapy. At a dose of 7.5 mg, the median lowest wbc, neutrophil, and platelet counts were 2.9, 1.4, and 203 $\times$ $10^3$/mm$^3$, respectively. At a dose of 10–12.5 mg, the median lowest wbc, neutrophil, and platelet counts were 2.8, 1.3, and 229 $\times$ $10^3$/mm$^3$, respectively. The lowest wbc and neutrophil counts occurred by Days 8–10 of therapy, whereas the lowest platelet counts occurred by Days 5–7.

Other toxic effects associated with the administration of vindesine are shown in table 1. Gastrointestinal and neurologic side effects, primarily in the form of nausea, vomiting, constipation, paresthesias, and weakness, were more common and became dose-limiting. Transient paralytic ileus occurred in six patients; one patient at 4 mg, four at 7.5 mg, and one at 12 mg. In general, toxic effects were observed more frequently at the higher doses and in patients with prior vinca alkaloid therapy. However, side effects associated with doses of 7.5 mg appeared tolerable. Significant alterations in biochemical parameters of renal and hepatic functions as well as those of serum electrolytes, that could be attributed to vindesine therapy, were not observed.

The antitumor efficacy of vindesine was evaluated in 60 patients who survived > 2 weeks and were able to receive a second course of chemotherapy. There were two partial remissions (PR), 12 disease stabilizations (including four minor responses), and 46 disease progressions. The two PRs occurred among 15 patients with acute leukemia (one each with acute lymphoblastic and myeloblastic leukemias), in spite of extensive prior chemotherapy which included vincristine. Minor responses were observed in two of 13 patients with renal cell cancer, in one of four patients with squamous cell lung cancer, and in one of one patient with esophageal cancer. Responses were brief, with a median duration of 12 weeks.

## DISCUSSION

The results of this study indicate that vindesine shares some of the favorable and unfavorable features of the other two vinca alkaloids. Like the parent compound, vinblastine, vindesine produces transient dose-related neutropenia and different

degrees of gastrointestinal toxic effects which include constipation and paralytic ileus. Contrary to what was expected from preclinical studies (2), and in similarity to vincristine, the administration of vindesine was associated with clinical manifestations of peripheral neuropathy. However, it appeared that the severity of these manifestations was less than could be expected from standard vinca alkaloids.

Total vindesine doses of 7.5–10 mg repeated at 2-week intervals were well-tolerated by most patients. If the mean body surface area of the patients treated is taken into account (1.85 $m^2$), these doses of vindesine would correspond to approximately 4–5 mg/$m^2$ given every 2 weeks. The toxic effects at these doses were of moderate degree and manageable. However, patients with extensive prior myelosuppressive chemotherapy were more prone to neutropenia after vindesine therapy. Patients with prior vincristine therapy were twice as likely to develop peripheral neuropathy. Accordingly, we recommend that for future phase II studies with vindesine, an initial dose of 4–5 mg/$m^2$ (approximately 7.5 mg) be given at 2-week intervals to good-risk patients (those who have received no prior chemotherapy), and a reduced dose of 3 mg/$m^2$ be given to poor-risk patients (those who have received extensive chemotherapy).

The antitumor activity of vindesine in man cannot be adequately judged from the results of this study in view of its phase I nature. However, the activity of this agent in several patients with extensive chemotherapy, including vincristine, is noticeable.

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# Broad Phase II Study of Vindesine[1,2,3]

Manuel Valdivieso,* Agop Y. Bedikian, Gerald P. Bodey, and Emil J Freireich[4]

Brief Reports and Preliminary Communications


## SUMMARY

**A broad phase II study of vindesine was conducted in 79 adults with advanced malignancies. Most patients had solid tumors, particularly adenocarcinomas (59%), and 67 patients had received prior chemotherapy which included vincristine in 24 patients and vinblastine in one. Depending on tolerance to previous therapy, patients received vindesine at doses of 3–4 mg/m² administered iv at 2-week intervals. Objective tumor regressions were seen in eight of 36 patients (one complete response, one partial response [PR], and six minor responses) with colorectal cancer, one of four patients (PR) with squamous cell cancer of the esophagus, one of four patients (PR) with malignant melanoma, and one of two patients (PR) with lymphoma. Five of 11 responding patients had received vincristine previously. Toxic effects were manageable. The results of this study justify in-depth, disease-oriented phase II evaluations of vindesine, regardless of prior therapy with other vinca alkaloids.**


[Cancer Treat Rep 65:877–879, 1981]

Vindesine is a newer vinca alkaloid derivative developed from vinblastine sulfate and introduced into clinical use because of its wide spectrum of antitumor activity in experimental systems and the potential of being less neurotoxic than vinblastine and vincristine (1,2). Phase I clinical studies have identified manageable dose-limiting myelosuppressive and neurologic toxic effects as well as hints of antitumor activity in a variety of tumors (3–6). We therefore conducted a broad phase II study of vindesine to further define its potential usefulness prior to conducting in-depth, disease-oriented phase II trials.

## PATIENTS AND METHODS

Seventy-nine adult cancer patients were the subjects of this study. Twelve patients had received no prior chemotherapy; all others were extensively pretreated. Signed informed consent was obtained according to institutional policies. Vindesine was obtained from Eli Lilly and Co, Indianapolis, IN.

Vindesine was given iv at an initial dose of 4 mg/m² in 100 ml of 5% dextrose solution over 30 minutes and repeated every 2 weeks. Patients with extensive prior chemotherapy received an initial dose of 3 mg/m². During subsequent treatments, doses were increased or decreased by 20% according to patient tolerance.

Cbcs and SMA-100 determinations were performed weekly in most patients. Appropriate radiologic and radioisotopic examinations were usually performed after every two to three treatment courses. Tumor measurements were recorded before each treatment course. Standard criteria were used to evaluate tumor response.

## RESULTS

Seventy-nine adult patients (42 males and 37 females) were entered in this study. Their median age was 57 years (range, 15–75). Patient malignancies included: colorectal carcinoma (36 patients); breast carcinoma (ten); renal cell carcinoma (seven); squamous cell carcinoma of the lung (six); squamous cell carcinoma of the esophagus (five); malignant melanoma (four); lymphoma (two); squamous cell carcinoma of the bladder (two); squamous cell carcinoma of the head and neck (two); and lymphoepithelioma, endometrial carcinoma, adenocystic

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[4]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

*Reprint requests to:* Dr. Manuel Valdivieso, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

carcinoma of the palate, adrenal carcinoma, and acute myeloblastic leukemia (one each). Twelve patients had received no prior chemotherapy (seven patients with renal cell carcinomas and five patients with squamous cell carcinomas of the esophagus). Among the remaining 67 patients, prior treatment included vincristine in 24 patients and vinblastine in one.

Seventy-one patients who received more than one course of vindesine were considered evaluable for response (table 1). Overall, there were one complete remission (CR) and four partial remissions (PR), plus six minor responses. Five of the 11 patients who achieved regressions had previously received vincristine. Tumor regressions were equally frequent in soft tissue or visceral sites. In the largest group in this study, ie, patients with colorectal cancer, a CR was achieved in a patient with metastatic disease to the liver and prior 5-FU and vincristine therapy. Response consisted of disappearance of hepatomegaly and normalization of the liver scan, abdominal ultrasound, plasma CEA, and liver enzyme examinations. The response continues at 9+ months. The PR occurred in a patient who achieved a > 50% regression of multiple pulmonary metastases associated with reduction of plasma CEA levels. This response lasted for 3 months.

The hematologic toxic effects encountered during this study resembled those observed in our initial phase I study (7). They consisted primarily of a moderate degree of neutropenia, with thrombocytopenia being less common. In 104 evaluable treatment courses, the median lowest counts and ranges (expressed as cells $\times 10^3/mm^3$) were: leukocytes, 2.9 (1.2–7.2); neutrophils, 1.8 (0.1–5); and platelets, 200 (42–602). The nadir was on Day 11 for leukocytes and neutrophils and on Day 7 for platelets. Eighty-seven percent of patients developed other drug-related toxic effects which were usually moderate. These included peripheral neuropathy (paresthesias) (35% of patients), constipation (35%), nausea and vomiting (35%), weakness (30%), fever (30%), stomatitis (22%), alopecia (17%), phlebitis (13%), diarrhea (4%), and myalgias (4%). Peripheral neuropathy appeared cumulative and clinically evident after three courses of therapy. It became severe in two patients who developed bilateral foot drop after doses of 6 $mg/m^2$. Electromyographic examinations conducted in six patients who developed clinical signs of peripheral neuropathy revealed different degrees of delayed peripheral nerve conduction. Overall, the manifestations of drug toxicity in this study were of such a degree that only one third of the treatment courses required dose reduction. Of the remaining treatment courses, 57% did not require dose modification and in 30%, dose escalation was possible.

## DISCUSSION

This broad phase II study of vindesine demonstrated that this newer vinca alkaloid derivative possesses antitumor activity in a variety of human malignancies, in spite of extensive prior chemotherapy. Of note is the activity that vindesine had in patients with advanced colorectal cancer where one CR, one PR, and six minor responses were achieved in a group of 36 patients. Four responses (one CR and three minor responses) were observed among ten patients previously treated with vincristine-containing regimens. PRs were also observed in one of four patients with squamous cell cancer of the esophagus, in one of four with malignant melanoma, and in one of two with non-Hodgkin's lymphoma. The combined results of our phase I (7) and II studies with vindesine indicated possible activity as well in acute leukemia, renal cell carcinoma, and squamous cell carcinoma of the lung. Other studies support these observations (8–10).

TABLE 1.—Response in evaluable patients

| Diagnosis | No. of patients | Complete response | Partial response | Stable disease | Disease progression | Duration of response (wks) |
|---|---|---|---|---|---|---|
| Colorectal carcinoma | 36 | 1 | 1 | 14 (6 minor responses) | 20 | 28+, 32+ |
| Breast carcinoma | 9 | — | — | 4 | 5 | |
| Renal cell carcinoma | 6 | — | — | 3 | 3 | — |
| Squamous cell carcinoma, lung | 5 | — | — | — | 5 | — |
| Squamous cell carcinoma, esophagus | 4 | — | 1 | 2 | 1 | 12 |
| Malignant melanoma | 4 | — | 1 | — | 3 | 2 |
| Lymphoma | 2 | — | 1 | — | 1 | 32 |
| Squamous cell carcinoma, head and neck | 2 | — | — | — | 2 | — |
| Others | 3 | — | — | 3 | — | — |
| Total | 71 | 1 | 4 | 26 (6 minor responses) | 40 | |

Of particular interest was the occurrence of remissions in patients with long-term exposure to vincristine, suggesting the possible lack of clinical cross-resistance between vindesine and vincristine. Observations along these lines have recently been reported (8). Manifestations of drug toxicity were manageable and consisted predominantly of neutropenia and peripheral neuropathy.

Vindesine is a newer vinca alkaloid derivative capable of producing tumor remissions in heavily pretreated patients with hematologic malignancies and solid tumors. The activity of vindesine in patients previously exposed to vincristine suggests the lack of clinical cross-resistance between these compounds and adds to the potential importance of vindesine. The results of this study justify in-depth, disease-oriented phase II studies of vindesine, with special emphasis on comparative trials with the parent compound vinblastine.

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# Phase II Trial of Anguidine in Patients With Sarcomas Unresponsive to Prior Chemotherapy: A Southwest Oncology Group Study[1,2]

Brief Reports and Preliminary Communications

J. Tate Thigpen, Clarence Vaughn, and W. J. Stuckey[3,*]

Therapy for advanced soft tissue and bone sarcomas has centered on the drug doxorubicin. Relatively few agents other than doxorubicin have demonstrated efficacy against sarcomas. This lack of effective drugs has hampered efforts to develop truly active drug combinations for the treatment of advanced sarcomas. As part of an ongoing effort to identify new active agents in sarcoma therapy, the Southwest Oncology Group elected to study anguidine as a single agent in therapy for advanced sarcomas.

## MATERIALS AND METHODS

At 13 member institutions of the Southwest Oncology Group, 27 adult patients (median age, 52 years; range, 15–76) with biopsy-proven metastatic sarcoma were evaluated with regard to history of prior treatment, performance status, extent of metastatic disease, hematologic parameters, and liver and renal functions. Tumor histology (table 1) included a variety of soft tissue and bony types. Most patients had received extensive prior therapy (table 1), and none started receiving anguidine until recovery from prior therapy was complete. Most patients were symptomatic and partially bedridden and had extensive metastatic disease when they entered the study. All were required to have wbc counts > 3000/μl, platelet counts > 100,000/μl, and normal BUN, creatinine, SGOT, and bilirubin.

Of the 27 patients entered in the study, one did not receive any drug and one was lost to followup.

TABLE 1.—Characteristics of the 25 patients studied

| Characteristic | No. of patients |
|---|---|
| Histologic type | |
| Soft tissue sarcomas | 22 |
| Leiomyosarcoma | 10 |
| Rhabdomyosarcoma | 4 |
| Epithelioid sarcoma | 3 |
| Neurofibrosarcoma | 2 |
| Other | 3 |
| Bony sarcomas | 3 |
| Prior treatment | 25 |
| Surgery | 23 |
| Radiotherapy | 16 |
| Chemotherapy | 24 |
| 1 regimen | 8 |
| 2 regimens | 8 |
| ≥ 3 regimens | 8 |
| Performance status (Karnofsky scale) | |
| 80%–100% | 3 |
| 50%–70% | 16 |
| < 50% | 6 |
| Extent of disease | |
| Local extension only | 10 |
| Pulmonary metastases | 12 |
| Hepatic metastases | 4 |
| Skeletal metastases | 4 |
| Other metastases | 5 |

The remaining 25 patients received anguidine (4.5 mg/m²/day) administered as a 4-hour infusion daily for 5 consecutive days and repeated every 21 days. Drug dose was increased by 1.5 mg/m²/day if little or no toxicity was observed, whereas doses were decreased by similar increments for severe or life-threatening toxicity. Wbc and platelet counts were performed weekly to determine the nadir.

A patient was considered to be evaluable for response if the patient received one drug course and survived 3 weeks, whereas the patient was evaluable for toxicity if he completed one drug course, regardless of subsequent survival. Response was defined as follows: *complete response*—the disappearance of all evidence of disease; *partial response*—a > 50% reduction in the product of the

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[3]This study was conducted by the Southwest Oncology Group. The following investigators participated in the study: *J. T. Thigpen* (University of Mississippi School of Medicine, Jackson); *C. Vaughn* (Providence Hospital, Southfield, MI); and *W. J. Stuckey* (Tulane University School of Medicine, New Orleans, LA).

*Reprint requests to:* Southwest Oncology Group, Operations Office, 3500 Rainbow Blvd, Suite 100, Kansas City, KS 66103.

perpendicular diameters of each measurable lesion; *disease progression*—a > 50% increase in the product of the perpendicular diameters of any measurable lesion or the appearance of any new lesion; and *stable disease*—a < 50% regression or progression of lesions. Responses or disease stabilization were recorded only if the observed status persisted for > 1 month.

## RESULTS

Of the 27 patients entered in the study, two were not evaluable for response. One never received the drug, and the other had no retrievable followup data after drug administration. Among the 25 evaluable patients, there were no objective responders. Twelve patients had stable disease for > 1 month, while 13 patients had disease progression. The 25 patients received a median of two drug courses (range, one to seven), with only one patient receiving more than three courses. Median survival of the 25 patients was 3 months.

Twenty-five of the 27 patients were considered evaluable for toxicity. The most significant adverse effect was myelosuppression, manifested in 16 of 25 patients as leukopenia and/or thrombocytopenia. Among the 14 patients who had leukopenia, a median wbc count nadir of 3750/μl (range, 700–3800) was observed on the median day, Day 17 (range, 3–49), following the start of therapy. Among the six patients who had thrombocytopenia, a median platelet count nadir of 55,000/μl (range, 26,000–94,000) was observed on the median day, Day 8 (range, 3–42), following the start of therapy. Only two patients had severe or life-threatening myelosuppression; there were no deaths related to bone marrow depression. Four of seven patients who received increased doses experienced mild or moderate leukopenia. The most commonly observed adverse effect was nausea and vomiting, seen in 16 of 25 patients. Three patients had nausea only, 12 had moderate nausea and vomiting, and one had severe vomiting. Other adverse effects included mild to moderate hypotension in six patients, hallucinations in one, erythema in one, stomatitis in one, and alopecia in one. No drug-related deaths were noted.

## DISCUSSION

Anguidine was selected for study by the Southwest Oncology Group because of evidence suggesting a unique mechanism of cytotoxicity in preclinical studies (1,2). Subsequent phase I trials (3,4) revealed that rapid infusion of the drug resulted in significant nausea and vomiting, somnolence, and erythema, while prolonged infusions over 8 hours resulted in myelosuppression as the dose-limiting adverse effect. To minimize these toxic effects, a 4-hour infusion was selected as the mode of drug administration in this trial.

The results of this trial of anguidine in patients with advanced sarcomas are disappointing in that no objective responses were observed among 25 patients. At best, a low order of efficacy would be expected in a patient population with as much prior treatment as the study population (23 of 25 patients had received extensive prior chemotherapy, and 14 had received as many as two to seven prior regimens), but the absence of any objective evidence of activity even in this patient population suggests that anguidine has no place in the treatment of sarcomas, at least at the dose and schedule tested.

Adverse effects observed most frequently were myelosuppression and nausea and vomiting, with both occurring in over one half of the patients. No drug deaths were observed, and most toxicity was mild to moderate. Hence, the dose and schedule used in this trial are tolerable and feasible.

In conclusion, anguidine in the dose and schedule tested appears to be inactive in the second-line therapy for advanced sarcomas.

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# Phase I Study on Bruceantin Administered on a Weekly Schedule[1,2]

Brief Reports and Preliminary Communications

Jean Liesmann,[3] Robert J. Belt,[3,4,*] Charles D. Haas,[3,4] and Barth Hoogstraten[3]

## SUMMARY

**Fifteen adult patients with advanced solid tumors received bruceantin at doses of 1.6–6.0 mg/m² iv for 30 minutes/week × 4, followed by a 2-week rest. The dose-limiting toxic effect was nausea and vomiting, which was more severe in patients with hepatic metastases or liver function abnormalities. Other sporadic toxic effects included fever, chills, malaise, alopecia, hypotension, thrombocytosis, and leukocytosis. Hematologic toxicity was insignificant. The recommended starting dose for phase II studies is 5 mg/m²/week × 4, every 6 weeks, with a reduction to 3 mg/m² for patients with hepatic metastases.**

[Cancer Treat Rep:883–885, 1981]

Bruceantin is a naturally occurring antitumor agent derived from the plant *Brucea antidysenterica*. The primary mechanism of action is the inhibition of protein synthesis (1,2).[5] Because of its activity against ip implanted murine leukemia P388, B16 melanoma, and L1210 leukemia, it was selected for clinical trials. The present phase I study describes our experience using a weekly iv dose.

## MATERIALS AND METHODS

### Drug Administration

Bruceantin was supplied by the National Cancer Institute (Bethesda, MD) in sterile 20-ml vials, each containing 3 mg of bruceantin. The drug was reconstituted with 1.5 ml of absolute ethanol and then further diluted with 13.5 ml of sodium chloride, USP, yielding 0.2 mg/ml of bruceantin. The drug was administered iv in an additional 250 ml of normal saline for 30 minutes/week × 4, followed by a 2-week rest.

The starting dose of bruceantin was 1.6 mg/m²/week × 4. Subsequent dose levels of 3.0, 5.0, and 6.0 mg/m² were evaluated in a minimum of three patients. Once significant toxic effects occurred, five to eight patients were treated at higher dose levels to more accurately determine the maximal tolerated dose of the weekly schedule. Four patients received one or more dose increases in the absence of dose-limiting toxic effects.

### Patients

Fifteen adult patients with histologically confirmed advanced malignancies refractory to conventional therapy received bruceantin after giving signed informed consent. All patients had adequate liver function, as defined by serum bilirubin ≤ 1.7 mg/100 ml, with alkaline phosphatase and SGOT ≤ 50% above normal values. All patients had adequate renal function, with serum creatinine ≤ 2 mg/100 ml, except one patient with chronic renal failure who had a creatinine level of 8.6 mg/100 ml. Pretreatment wbc counts were at least 3500/mm³ and platelet counts were > 100,000/mm³, except in one patient who had a wbc count of 2900/mm³. Patients were followed with weekly cbcs, platelet counts, and chemistry screens.

Diagnoses of patients treated with bruceantin included adenocarcinoma of the colon (seven), ovary (two), breast (one), and endometrium (one); non-small cell carcinoma of the lung (two); and squamous cell carcinoma of the head and neck (two). All patients had received previous treatment. Nine had had prior chemotherapy, and six had had both chemotherapy and radiation therapy. The median Karnofsky performance status was 60%, with a range of 30%–100%.

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[2]Supported in part by contract N01-CM-87208-26 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. J. Liesmann is a Clinical Fellow, and B. Hoogstraten is an American Cancer Society Professor of Medicine.

[3]Division of Medical Oncology, University of Kansas Medical Center, Kansas City.

[4]Veterans Administration Medical Center, Kansas City, MO.

[5]Clinical brochure: bruceantin. Prepared by the National Cancer Institute, Bethesda, MD, Jan 1978.

**Reprint requests to:* Robert J. Belt, MD, Division of Medical Oncology, University of Kansas Medical Center, 39th and Rainbow Blvd, Kansas City, KS 66103.

TABLE 1.—Correlation of gastrointestinal toxicity and liver function

| Dose (mg/m²) | Liver metastases* | | | No liver metastases* | | |
|---|---|---|---|---|---|---|
| | Total No. of patients | No. of patients with— | | Total No. of patients | No. of patients with— | |
| | | Abnormal liver function tests† | Severe nausea and vomiting‡ | | Abnormal liver function tests† | Severe nausea and vomiting‡ |
| 1.6 | 1 | 0 | 0 | 2 | 0 | 0 |
| 3.0 | 1 | 1 | 1 (1) | 3 | 0 | 0 |
| 5.0 | 3 | 1 | 2 (2) | 4 | 0 | 1 |
| 6.0 | 4§ | 3 | 3 (1) | 1 | 0 | 1 |

*Determined clinically by radionuclide or computerized tomographic scan or documented by laparotomy.

†SGOT, alkaline phosphatase, and bilirubin < 50% above normal.

‡Defined as inability to take food or liquids by mouth and experiencing > 10 episodes of vomiting in spite of antiemetics. Values in parentheses = No. of patients who refused further therapy.

§Each of 2 patients received 2 courses of bruceantin; they had the greatest degree of gastrointestinal toxic effects.

## RESULTS

Fifteen patients received 23 courses of bruceantin at doses of 1.6–6.0 mg/m² iv for 30 minutes/week × 4. Two courses were nonevaluable, one because of early non-drug-related death and one because of rapid disease progression resulting in termination of therapy. In addition, five courses were not completed because of severe vomiting that resulted in patient refusal to continue the drug. These courses were evaluated for gastrointestinal toxicity only. The 16 remaining courses were fully evaluable for the spectrum of drug-related toxic effects.

The dose-limiting toxic effect from bruceantin was nausea and vomiting. Emesis occurred at all doses but was consistently more severe at the higher dose levels. Four of seven courses given at a dose of 6 mg/m² resulted in severe emesis, and two of six courses given at a dose of 5 mg/m² resulted in severe gastrointestinal toxicity. Four patients (one who received a dose of 3 mg/m², two who received 5 mg/m², and one who received 6 mg/m²) refused further treatment after one to three doses because of severe vomiting. In general, patients who had moderate to severe emesis had a significant alteration in their activities and weight loss during the course of treatment because of the severity and duration of gastrointestinal symptoms.

There was a correlation between liver metastases or liver dysfunction and the severity of nausea and vomiting (table 1). All four patients who refused to continue treatment had liver metastases, often with mild abnormalities of liver function. There was no correlation between gastrointestinal toxicity and renal function, performance status, or any other recognizable parameter. The one patient with renal failure showed the same or a lesser degree of toxic effects, compared to others treated at the same dose.

Other sporadic toxic effects were seen. Two patients had severe malaise, chills, and fever for 4 days after treatment. Two patients had headaches, one had phlebitis above the injection site, three had moderate alopecia, and one reported lightheadedness. One patient treated at a dose of 5.0 mg/m² became hypotensive for 4 hours but responded to fluids and recovered without incident. She subsequently received nine more doses without developing hypotension. At a dose of 5 mg/m², one patient developed thrombocytosis and leukocytosis 1 week after the first dose; this persisted until his death 4 weeks later.

Three patients had mild elevations in liver enzymes (SGOT and alkaline phosphatase) at doses of 5.0–6.0 mg/m². With the exception of one patient who died with sepsis, the enzyme levels returned to normal despite continued treatment with bruceantin.

Of the 16 courses evaluable for hematologic toxicity, only one resulted in a wbc count < 4000/mm³. That patient received a dose of 5.0 mg/m² when her baseline wbc count was 3600/mm³, and subsequently she had a wbc count of 2900/mm³ on Day 8. She continued to receive weekly bruceantin treatments, and her wbc count returned to 3400/mm³ the following week. At a dose of 6.0 mg/m², her platelet count decreased from 100,000 to 85,000/mm³ on Day 22, with recovery despite continued treatment.

There was stabilization of disease for 14 weeks in only one patient with adenocarcinoma of the uterus. There was no other significant stabilization of disease or objective response.

## CONCLUSION

In the present study, bruceantin was evaluated at iv doses of 1.6–6.0 mg/m²/week × 4, followed by a 2-week rest. This drug was poorly tolerated; patients had excessive nausea, vomiting, and malaise at doses ≥ 5.0 mg/m². A correlation between the degree of gastrointestinal toxicity and the presence of liver metastases was seen. Although many patients with hepatic metastases had mild (< 50% above normal) elevations in bilirubin, alkaline phosphatase, and/or SGOT, the number of patients with any given pattern of liver function abnormalities was too few to correlate with gastrointestinal toxic effects.

Two other phase I studies of bruceantin were recently completed using daily doses for 5 days, repeated every 3 weeks, and a weekly schedule identical to ours (3,4). Both studies reported the dose-limiting toxic effect to be hypotension. Gastrointestinal toxic effects were common in both studies but were not severe or dose-limiting in most patients (3,4). Using daily doses for 5 days, Bedikian et al (3) observed nausea and vomiting in 47% of courses at doses of 3.0–4.5 mg/m²/day. The severity and duration of nausea and vomiting were greater when higher doses were administered. Since gastrointestinal toxicity is dose-related and apparently not cumulative, the difference in the degree of nausea and vomiting reported by Bedikian et al and that observed in our study probably relates to the higher single-day dose used in the weekly schedule. Increased toxicity, manifested by myelosuppression and hypotension, was associated with hepatic dysfunction in the study reported by Bedikian et al.

Garnick et al (4) used the same schedule as that in our study but reported gastrointestinal toxicity in only 29%–67% of doses administered in the range of 5.0–8.5 mg/m²/week. This difference may be accounted for by the absence of hepatic dysfunction in their patients. Despite some differences in the severity and overall incidence of drug-related toxic effects, the dose of 5.5 mg/m²/week recommended by Garnick et al for phase II testing is very close to the 5.0 mg/m²/week which we recommended for a starting dose in phase II studies. We also recommend a gradual dose increase in the absence of toxicity. If there is significant emesis, a dose reduction to 3 g/m² is indicated. Because of the more severe gastrointestinal toxicity associated with liver dysfunction, a starting dose of 3 mg/m²/week should be used for patients with known liver metastases or clinical evidence of hepatic dysfunction. Gradual dose increases are appropriate for patients who do not have moderate to severe toxic effects.

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# Phase I Study of VP-16-213 (Etoposide) Administered as a Continuous 5-Day Infusion[1]

Brief Reports and Preliminary Communications

J. Lokich* and J. Corkery[2]

## SUMMARY

**Twenty-four patients with advanced malignancy received 30 courses of VP-16-213 delivered by a protracted venous infusion for a minimum of 5 days or 120 hours in a phase I study to determine the maximum tolerated dose. Toxic effects included fluid overload (due to the necessary use of normal saline as the drug delivery vehicle), stomatitis, and marrow suppression with leukopenia and thrombocytopenia. Except for the stomatitis, gastrointestinal effects were virtually absent. The dose-limiting toxic effect was marrow suppression observed at a dose of 60 mg/m²/day × 5 days (300 mg/m²/course). Six of seven patients treated at this dose developed marrow suppression: 50% (three of six patients) had life-threatening leukopenia associated with fever only (one patient), sepsis (one), and septic death (one).**

[Cancer Treat Rep 65:887–889, 1981]

VP-16-213 (Etoposide), a derivative of podophyllotoxin, is an antitumor compound with significant activity in a broad spectrum of tumors including lymphoma, leukemia, small cell lung carcinoma, and ovarian and testicular cancers (1). Unlike the podophyllotoxin parent compound, VP-16-213 does not inhibit the microtubular assembly but rather may induce DNA breaks directly (2,3) and arrest cells in the late S or $G_2$ phase of the cell cycle (4). Pharmacology studies of VP-16-213 reveal a biexponential decay curve, with a mean half-life of approximately 11 hours (5). In preclinical studies, particularly in the L1210 leukemia system, marked schedule dependency was demonstrated (6); in clinical studies in small cell carcinoma of the lung, schedule dependency was observed, with a doubling of the response rate in patients receiving daily treatment as opposed to weekly treatment (1).

A phase I trial of constant-infusion VP-16-213 was initiated on the basis of cytokinetic mechanism of action, pharmacology, and preclinical and clinical data, indicating that continuous drug exposure may be advantageous in promoting therapeutic effects.

## MATERIALS AND METHODS

All patients entered in the phase I continuous-infusion trial of VP-16-213 had a histologic diagnosis of metastatic malignancy and were considered candidates for investigational therapy in that standard forms of therapy were exhausted or were not known. All patients had a life expectancy of > 4 weeks and patients receiving prior chemotherapy had a minimum lapse of 4 weeks before entry in the trial.

VP-16-213 was delivered in a minimum volume of 1–2 liters of normal saline/24 hours. The drug was stable for at least 6–8 hours; therefore, the infusing solution was reconstituted to be run over 8-hour intervals and the drug was delivered by constant pump infusion. All patients received consecutive infusion for 120 hours or 5 days.

Subsequent to the treatment, patients were monitored weekly by cbc and platelet count for marrow suppression. Tumor parameters were monitored by appropriate studies including radiographs at monthly intervals. Therapy was repeated at 4-week intervals if no tumor growth was observed in the interim and if drug tolerance was optimal. Dose escalations were made for subsequent courses based on interim blood cell counts and nadir levels. Dose escalation between patients was determined by a modified Fibonacci scheme. The initial dose for patient entry was 20 mg/m²/day, approximately one third of the total dose employed in clinical studies of daily bolus treatment for 5- or 7-day therapy. Gastrointestinal toxicity was graded as follows: 1 = transient nausea without anorexia; 2 = intermittent vomiting with meals only; and 3 = protracted daily vomiting. Sto-

[1]Received May 22, 1980; revised Oct 27, 1980, accepted Nov 24, 1980.

[2]Section of Medical Oncology, Department of Medicine, New England Deaconess Hospital, Harvard Medical School, Boston, MA.

*Reprint requests to: Jacob J. Lokich, MD, Section of Medical Oncology, Department of Medicine, New England Deaconess Hospital, Harvard Medical School, 185 Pilgrim Rd, Boston, MA 02215.

TABLE 1.—Dose escalation and marrow toxicity in phase I study of continuous-infusion VP-16-213

| Dose ($mg/m^2$/day) | No. of patients | Wbc count ($cells/mm^3$) | | Platelet count ($cells/mm^3$) | |
|---|---|---|---|---|---|
| | | < 1000 | 1000–3000 | < 50,000 | 50,000–100,000 |
| 20 | 3 | 0 | 0 | 0 | 0 |
| 30 | 2 | 0 | 0 | 0 | 0 |
| 40 | 2 | 0 | 0 | 0 | 0 |
| 50 | 2 | 0 | 0 | 0 | 0 |
| 60 | 7 | 3 | 3 | 3 | 1 |
| 70 | 7 | 2/4* | 3/4* | 1/4* | 1/4* |
| 80 | 2 | 0 | 1/1* | 0 | 0 |

*Denominator = only patients for whom weekly blood cell counts were available.

matitis was graded from 0 to 4+ based on the quantitative extent of ulceration and mouth pain.

Tumor response was determined using standard definitions of response. A partial response was defined as a > 50% decrease in the product of the perpendicular diameters of all discretely measurable lesions. A complete response was defined as a complete regression of all measurable disease for a minimum period of 1 month, with concomitant symptomatic improvement.

## RESULTS

Twenty-four patients were entered in the study and received a total of 30 courses: 21 patients received one course and one patient each received two, three, and four courses at escalated or stable dose levels. Patients ranged in age from 20 to 80 years. The tumor categories were as follows: lung cancer (eight patients, five with oat cell carcinoma); leukemia-lymphoma (four); breast cancer (two); tumor of unknown origin (two); and melanoma, hepatoma, renal cell carcinoma, pancreatic cancer, colon cancer, multiple myeloma, renal/pelvis tumor, and nasopharyngeal carcinoma (one each).

A summary of the dose escalation and observed toxicity is detailed in table 1. Doses were incrementally increased at 10 $mg/m^2$/day between groups composed of two or three patients. The initial observations of toxicity were achieved at a dose of 60 $mg/m^2$/day (300 $mg/m^2$/course) in poor-risk patients and therefore patient entry was continued to 70 and 80 $mg/m^2$/day. Marrow suppression was observed in six of seven patients at a dose of 60 $mg/m^2$/day; three patients had granulocytopenia (< 500 $cells/mm^3$) and three had thrombocytopenia (< 50,000 $platelets/mm^3$). This degree of marrow suppression led to fever only (one patient), fever and sepsis (one), and septic death (one). All six patients had received major prior radiation therapy (four patients) or chemotherapy (four), or they had bone metastases (three) or liver disease (two). Good-risk patients with minimal prior therapy tolerated the higher dose levels of 70–80 $mg/m^2$/day, although leukopenia was observed in four of four evaluable patients at 70 $mg/m^2$/day and the one evaluable patient at 80 $mg/m^2$/day. The duration of leukopenia and/or thrombocytopenia was 5–14 days and the median was 8 days.

Stomatitis or oral ulceration was observed in four patients receiving a dose of ≥ 60 $mg/m^2$/day. In two of the four patients, prior radiation to the head and neck area suggested that radiation sensitization may have played a role in the resulting stomatitis. The stomatitis developed by Day 5 of therapy and was most severe (grade 4) in patients irradiated previously. Gastrointestinal toxicity in the form of nausea, vomiting, or diarrhea was not observed in any patient.

Dependent edema and congestive heart failure were observed as a consequence of the administration of normal saline in large volumes to maintain the stability of the drug during the 24-hour infusion. Patients were carefully monitored and treated with cardiac glycosides and diuretics, as necessary, but treatment was never interrupted.

No evidence of pulmonary, cutaneous, hepatic, or renal toxicity was noted. Cumulative toxicity could not be evaluated because the majority of patients received only a single course of therapy with VP-16-213 due to progressive disease or excessive toxicity.

Responses were observed in two of two patients with lymphoma and in one patient with adenocarcinoma of the lung. Minor responses were observed in one patient who experienced relief of bone pain and in one patient with leukemia in whom the peripheral blast cell count decreased but the bone marrow remained unchanged. All responses were observed at a dose ≥ 60 $mg/m^2$/day. Patients responding to therapy were subsequently treated with the combination of cyclophosphamide and VP-16-213.

## DISCUSSION

VP-16-213 and another derivative of podophyllotoxin, VM-26, are promising new agents in cancer chemotherapy and have been the subject of comprehensive reviews within the past year (7–9). Preclinical studies have suggested a potential superiority for constant-infusion programs, and in clinical studies daily schedules have been superior to weekly intermittent schedules, at least in small cell carcinoma of the lung (1). In spite of such data, protracted, continuous venous infusion studies of VP-16-213 have not been previously reported.

The dose-limiting toxicity for VP-16-213 in previous reports was leukopenia, generally without thrombocytopenia (10–12). Fifty percent of the patients had gastrointestinal toxic effects, particularly nausea, vomiting, and diarrhea, at doses of 300 to 375 mg/m². In the original three phase I trials by Creaven et al (10), Nissen et al (11), and Eagan et al (12), in which VP-16-213 was delivered iv, the maximum tolerated dose was 290–375 mg/m². In these bolus or pulse infusion studies, the dose schedule varied from 3 to 7 consecutive days, repeated at 3-week intervals.

In the protracted continuous-infusion delivery program reported here, marrow suppression was dose-limiting, manifested by both leukopenia and thrombocytopenia. The maximum tolerated dose for VP-16-213 by constant infusion was 60 mg/m²/day (total cumulative dose, 300 mg/m²). The starting dose for good-risk patients (ie, patients with good marrow reserve, without bone or marrow involvement and without prior irradiation to marrow-bearing areas) may be as high as 80 mg/m²/day (total dose, 400 mg/m²). In poor-risk patients, maximum initial dose should not exceed 50 mg/m²/day for 5 consecutive days, or a maximum total dose of 250 mg/m². Additional dose modification in patients with biliary obstruction may be necessary because VP-16-213 is secreted by the biliary tract.

The possibility that VP-16-213 is a radiation sensitizing agent has been previously reported and is confirmed by the observation of severe stomatitis in two patients who had received prior oral radiation therapy (13). This was not a consistent observation, however, in that cutaneous areas exposed to prior radiation did not develop a recall reaction following VP-16-213 therapy. Stomatitis was only observed at higher dose levels.

Although a recent study of a large group of patients has suggested that VP-16-213 has a narrow spectrum of activity (14), the drug schedule did not include protracted venous infusion treatment. Three responses were observed in this study, two in a tumor type known to respond to VP-16-213. It is possible that the effectiveness of VP-16-213 may be expanded in terms of the tumor spectrum by this protracted venous infusion program and a phase II trial has been initiated to evaluate such a possibility. In addition, multiple-drug programs combining cytarabine, cyclophosphamide, or doxorubicin with VP-16-213 may improve the effectiveness of this unique antitumor agent.

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# Doxorubicin Hypogastric Artery Infusion Combined With Hyperthermia Therapy for Transitional Cell Carcinoma of the Bladder[1]

Brief Reports and Preliminary Communications

Stephen C. Jacobs,* Frank E. Maddison, and Russell K. Lawson[2]

## SUMMARY

**Six patients with bulky stage C bladder cancer underwent doxorubicin hypogastric artery infusion combined with local bladder hyperthermia as a preoperative adjunct to radical cystectomy. The regimen was well-tolerated. Extensive tumor necrosis on the bladder luminal surface was seen during histopathologic examination.**

[Cancer Treat Rep 65:891–893, 1981]

Doxorubicin and hyperthermia are synergistic in both in vivo and in vitro tumor model systems (1,2). Doxorubicin is an active agent against transitional cell carcinoma when given topically (3), iv (4,5), or intra-arterially (6). Bladder hyperthermia alone results in some regression of transitional cell carcinoma (7). A phase I study was instituted to evaluate the combination of hypogastric artery infusion of doxorubicin and bladder hyperthermia as a preoperative adjunct to radical cystectomy for transitional cell carcinoma of the bladder.

## MATERIALS AND METHODS

Six patients with bulky clinical stage C bladder cancer have undergone treatment (table 1). Diagnosis was established by transurethral biopsy. Staging included bimanual examination under anesthesia, iv pyelogram, pelvic computerized tomographic scan, chest tomography, and bone and liver-spleen scans.

Via a percutaneous femoral artery route, bilateral arterial catheters were placed at the trifurcation of the hypogastric arteries just distal to the origin of the superior gluteal arteries. Doxorubicin (40–60 mg/m$^2$) was infused with a Harvard pump over 48 hours.

Bladder hyperthermia was achieved by pump[3] circulation of heated water through a 24-French three-way Foley catheter. The temperature used in the first patient was 39°C and was increased with each succeeding patient to a level of 44°C. Inflow and outflow temperatures were continuously monitored with thermistors.[4] Outflow resistance was adjusted to maintain the bladder in an approximately half-filled state (200 ml). The hyperthermia was maintained for a total of 96 hours.

Radical cystectomy was performed 3 weeks after the initiation of treatment.

## RESULTS

Pelvic angiography at the time of arterial catheter placement revealed relatively avascular tumors, as is expected in transitional cell carcinoma.

No complications from the indwelling arterial catheters occurred. Doxorubicin produced leukopenia in one patient (nadir wbc count, 825/mm$^3$). Alopecia occurred in all six patients. Bladder hyperthermia caused only mild bladder discomfort in two patients which was relieved by oral anticholinergics. No other urinary tract complications occurred.

Cystoscopy 1 and 2 weeks after doxorubicin/bladder hyperthermia treatment revealed generalized necrosis of the tumor surface. Normal bladder mucosa became edematous; one bladder developed a pseudomembrane over the surface of the normal urothelium. Five patients underwent radical cystectomy with ileal conduit urinary diversion 3 weeks after treatment. One patient also underwent exploratory laparotomy and external-beam radiation therapy (5000 rads) before cystectomy. Operative blood loss averaged 6 units.

At pathologic examination, two patients had necrotic common iliac lymph nodes which proved to

[1]Received July 7, 1980; revised Jan 7, 1981; accepted Jan 27, 1981.

[2]Departments of Urology and Radiology, The Medical College of Wisconsin, Milwaukee.

*Reprint requests to:* Stephen C. Jacobs, MD, 9200 W Wisconsin Ave, Milwaukee, WI 53226.

[3]Thermomix 1420, B. Brown Co.

[4]Tele-thermometer, Yellow Springs Instruments.

TABLE 1.—Patients with bulky clinical stage C bladder cancer

| Patient age (yrs)/sex | Bladder (°C) | Doxorubicin ($mg/m^2$) | Other treatment | Pathologic stage | Pathologic effects* | Survival (wks)† |
|---|---|---|---|---|---|---|
| 65/M | 39 | 40 | | C | Perivesical fat necrosis | 54, NED |
| 57/M | 39.6 | 40 | 4500 rads postoperatively | D | Necrotic nodes | Died at 25 (TCC) |
| 58/F | 41 | 40 | 5000 rads preoperatively | D | Necrotic nodes | Died at 23 (TCC) |
| 66/M | 41 | 60 | | C | | Died at 34 (TCC) |
| 77/M | 44 | 60 | | C | | Died at 12 (CVA) |
| 56/M | 44 | 60 | | D (prostate invasion) | Bladder, pseudo-membrane | 18, NED |

*All bladders showed extensive necrosis of tumor on the luminal surface.
†NED = no evidence of disease; TCC = transitional cell carcinoma; CVA = cardiovascular accident.

contain some viable transitional cell carcinoma. One patient had prostatic invasion. All primary tumors had extensive necrosis, particularly on the bladder luminal surface. Peripherally, however, all patients showed viable tumor. The normal bladder mucosa remained viable in all patients. An amorphous pseudomembrane formed over the normal mucosa in one case. The submucosa became markedly edematous. In areas near the tumor, detrusor muscle fibers were undergoing myolysis. In areas away from the tumor, muscle remained normal. Extensive perivesical fat necrosis was seen in one case.

Two of the six patients are alive with no evidence of disease at 18 and 54 weeks. One patient died of unrelated causes at 12 weeks without evidence of disease at autopsy. Three patients died of transitional cell carcinoma at 23–24 weeks. All had metastases in the lung and liver. One patient had persistent disease in the abdomen and pelvis and two had no disease in the pelvis.

## DISCUSSION

Homogeneous heating of a tumor depends on the tumor volume, the temperature applied, and the thermal dissipation via blood supply (8). The bladder cancers treated were large and heat was applied to the luminal surface only. The temperature was low enough to cause only mild changes in the normal bladder tissue. The tumor blood flow may have initially been less than the normal tissue. Hyperthermia causes an increased circulation in normal tissues and enhancement of heat dissipation, but this does not occur in tumor tissue (9). Heat would have been dissipated less rapidly if the tumor blood supply could be temporarily interrupted. Monitoring of the temperature achieved was done by measuring the outflow temperature. Placement of needle thermistors percutaneously was not done for fear of seeding of the needle tracts. A more homogeneous distribution of hyperthermia would be obtained by other external hyperthermia modalities but these subject adjacent structures (ie, rectum) to hyperthermia. Exposures to hyperthermia must then be limited to shorter time periods. The pump circulation method employed in the study did not elevate rectal temperature.

Doxorubicin infusion of the hypogastric artery for the palliative treatment of extensive pelvic cancer has previously been performed (6). During such infusions, significant delivery of doxorubicin to the buttocks and rectum occurs. The arterial catheters in this study were placed beyond the superior gluteal arteries to diminish doxorubicin perfusion of the buttocks. No rectal complications occurred at the doses employed.

The extensive tumor necrosis seen pathologically was primarily on the luminal surface, indicating that possibly lack of homogeneous heating resulted in suboptimal treatment of tumor on the serosal surface. Increasing the temperature, the doxorubicin dose, or the time interval to cystectomy, may decrease the amount of residual viable tumor. The mechanism for necrosis of metastatic common iliac lymph nodes in two patients is not known. Necrotic metastatic nodes are not seen in untreated bladder cancer. However, the nodes seen in these two patients were clearly not in the field of direct arterial infusion nor could the local bladder hyperthermia have altered their temperature directly. Systemic doxorubicin absorption is the most likely explanation for the nodal effects.

Newer adjuvant therapies to radical cystectomy are needed. While adjuvant radiation therapy has helped with control of pelvic disease, it has not decreased systemic failures and has, on occasion, made surgical resection more difficult. Potentially, arterial infusion adjuvant therapies might aid in both local pelvic and systemic control. The optimum preoperative drug, doses, and scheduling have yet to

be defined. Possibly even longer or multiple preoperative infusions would be more beneficial.

In conclusion, adjunctive preoperative hypogastric artery infusion of doxorubicin plus bladder hyperthermia is safe, well-tolerated, and merits further study to determine the upper limits of safety of both doxorubicin dose and hyperthermia temperature.

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# Progestin Therapy for Advanced Ovarian Cancer: A Phase II Eastern Cooperative Oncology Group Trial[1,2]

Brief Reports and Preliminary Communications

Robert E. Slayton,* Marcello Pagano, and Richard H. Creech[2]

Using a variety of criteria to define response, several investigators have observed response to progestin therapy for advanced ovarian cancer (1–7). Tobias and Griffiths (8) reported a cumulative response rate of 38% in 60 patients. They and others (9) have urged a controlled phase II trial of progestin therapy for ovarian cancer using standardized criteria to define response.

The following is a summary report of a phase II trial of high-dose progestin therapy for ovarian cancer initiated by the Eastern Cooperative Oncology Group (ECOG) in August 1977.

## MATERIALS AND METHODS

Selecting a high dose to ensure adequate blood levels, 1000 mg of medroxyprogesterone acetate (Depo Provera) was given weekly by im injection to 19 women with ovarian cancer no longer responding to surgery, radiation therapy, or conventional cytotoxic chemotherapy. Patients without measurable disease were ineligible. The drug was administered for at least 8 weeks, and longer if there was no evidence of tumor progression. Performance status (PS) was defined as follows (ECOG criteria): grade 0 = fully active, able to carry on all predisease activities without restriction (Karnofsky scale, 90–100); grade 1 = restricted in physically strenuous activity but ambulatory and able to do work of a light or sedentary nature, eg, light housework, office work (Karnofsky scale, 70–80); grade 2 = ambulatory, capable of all self-care, and out of bed > 50% of waking hours but unable to do any work (Karnofsky scale, 50–60); grade 3 = capable of only limited self-care, confined to bed or chair ⩾ 50% of waking hours (Karnofsky scale, 30–40); and grade 4 = completely disabled, incapable of any self-care, totally confined to bed or chair (Karnofsky scale, 10–20). Only patients with a PS of 0–3 were eligible, thus excluding patients who were completely bedridden. One patient had a PS of 0, nine had a PS of 1, five had a PS of 2, and four had a PS of 3. Adequate renal and hepatic functions (BUN < 25 mg/dl, creatinine < 1.5 mg/dl, bilirubin < 1.5 mg/dl, and SGOT < 60 IU/liter) were required. Patients with inadequate bone marrow function were not excluded. Eight patients were admitted to the trial with a wbc count < 4000/mm$^3$ and/or a platelet count < 100,000/mm$^3$. Age ranged from 30 to 70+ years (median, 54).

## RESULTS

Defining regression as a ⩾ 50% decrease in the sum of the products of the perpendicular diameters of measurable lesions and disease progression as a ⩾ 25% increase over the original measurements, or the appearance of new lesions, there were no responses. Only one patient remained stable (3 months) while receiving medroxyprogesterone acetate. The median survival of the 19 patients on medroxyprogesterone acetate was 2 months, with 75% of the patients dying within 4 months. Although severe vomiting was seen in one patient and bleeding in a second, neither of these adverse reactions could be related directly to medroxyprogesterone acetate administration. There was no other toxicity. As-

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[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (*R. E. Slayton*, CA-10948); Sidney Farber Cancer Institute, Boston, MA (*M. Pagano*, CA-23318); and American Oncologic Hospital, Philadelphia, PA (*R. H. Creech*, CA-18281). Other participating institutions include: Albert Einstein College of Medicine, Bronx, NY (CA-14958); Hahnemann Medical College, Philadelphia, PA (CA-13611); Abraham Lincoln School of Medicine, Chicago, IL; Jefferson Medical College, Philadelphia, PA (CA-14215); Johns Hopkins Oncology Center, Baltimore, MD (CA-16116); Mayo Clinic, Rochester, MN (CA-13650); University of Minnesota, Minneapolis (CA-20365); New York University Medical Center, NY (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); University of Rochester Cancer Center, NY (CA-11083); and Medical College of Virginia, Richmond (CA-10572).

**Reprint requests to:* Robert E. Slayton, MD, Section of Medical Oncology, Department of Internal Medicine, Rush Medical College, 1725 W Harrison St, Chicago, IL 60612.

suming the response rate to medroxyprogesterone acetate is as low as 20%, the probability of observing no response in 19 patients is 0.023. Any higher response rate would lead to an even smaller probability. Consequently, it is likely that the true response rate to medroxyprogesterone acetate is $< 20\%$.

## DISCUSSION

Although Tobias and Griffiths (8) cite a 38% cumulative response rate, only one of ten patients in the Jolles series (1) and three of 23 in the Ward series (5) had objective responses using modern response criteria. Malkasian et al (6) reported two responses in nine patients treated orally with 6,17a-dimethyl-6-dehydroprogesterone. Four years later, however, the same group reported only one short-term objective response in 19 patients treated with oral medroxyprogesterone acetate (10).

In this unselected population with advanced ovarian cancer refractory to conventional chemotherapy, progestin therapy did not have a beneficial effect. Further trials of progestin therapy for ovarian cancer should be limited to patients with endometrioid histology, with special emphasis on the relationship between progesterone receptor activity and response.

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# Phase II Trial With Cisplatin, Doxorubicin, and Cyclophosphamide (CAP) in the Treatment of Urothelial Transitional Cell Carcinoma[1,2]

Brief Reports and Preliminary Communications

M. Campbell,[3] L. H. Baker,[3] M. Opipari,[4] and M. Al-Sarraf[3,5,*]

## SUMMARY

**Patients with advanced transitional cell cancer of the bladder, urethra, or renal pelvis were treated with a combination of cyclophosphamide, doxorubicin, and cisplatin. Fifteen patients were evaluated, 12 of whom had cancer of the urinary bladder. Metastatic sites were bone (47% of the patients); lung (33%); pelvis (27%); liver (20%); nodes (7%); and brain (7%). Only two patients (13%) achieved a partial response (in measurable node and lung lesions) and survived 16 and 58 weeks. Three patients (20%) had minor responses (in measurable bone, pelvic mass, and bone and local lesions) and survived 8, 28, and 102+ weeks, respectively. Four patients had stable disease and six had disease progression, with a median survival of 4 weeks. Overall toxic effects were leukopenia (53% of the patients); thrombocytopenia (27%); nausea and vomiting (67%); alopecia (60%); and renal (13%). We concluded that the response rate to the combination of cyclophosphamide, doxorubicin, and cisplatin in transitional cell cancer of the urothelium was not superior to the reported response rate to a single agent (eg, cisplatin), and toxicity was greater.**

[Cancer Treat Rep 65:897–899, 1981]

Many chemotherapeutic agents have been used in the treatment of locally advanced or metastatic transitional cell carcinoma of the urothelium. Single-agent activity has been demonstrated for cyclophosphamide (1), doxorubicin (1–5), and, most recently, for cisplatin (5–9). Studies of murine tumors suggested that the combination of doxorubicin and cyclophosphamide may be synergistic (10,11). Initial clinical studies with the combination of chemotherapeutic drugs yielded varying response rates from 17% to 50% (12–14) and did not clearly prove superiority over single agents. Cisplatin appears to be the most effective single agent and has also been introduced into combination therapy (5,6,8,15,16). A trial of the three-drug combination suggested significant activity, with an overall response rate of 90% (17). Since the various combinations have met with mixed results and their superiority over single agents has not yet been demonstrated, we felt further evaluation of cyclophosphamide, doxorubicin, and cisplatin (CAP) in transitional cell carcinoma of the urothelium was warranted.

## MATERIALS AND METHODS

Patients with histologically confirmed transitional cell carcinoma of the urothelium who had measurable locally advanced or metastatic disease were entered in the study.

Pretreatment evaluation consisted of a history and physical examination, multiphasic blood chemistry studies, creatinine clearance determination, ECG, excretory urogram, and appropriate roentgenograms and scans, as indicated.

Patients having received prior systemic chemotherapy were not eligible. A BUN $< 20$ mg/100 ml, serum creatinine $< 1.5$ mg/100 ml, creatinine clearance $> 60$ ml/minute, bilirubin $< 1.5$ mg/100 ml, wbc count $> 4000/mm^3$, and platelet count $> 100{,}000/mm^3$ were required.

Patients were separated into "good-risk" and "poor-risk" groups, the latter being defined as those $\geq 65$

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[3]Department of Oncology, Wayne State University, and Harper-Grace Hospitals, Detroit, MI.

[4]Department of Medicine, Michigan State College of Osteopathic Medicine, and Detroit Osteopathic Hospital, Highland Park, MI.

[5]We thank Rose Marie Mason and Marie Schumacher for secretarial assistance.

*Reprint requests to: M. Al-Sarraf, MD, Department of Oncology, Wayne State University, 3990 John R St, Detroit, MI 48201.

years of age with extensive prior radiotherapy or those with poor bone marrow reserve.

On the first day of the schedule, patients received a minimum of 2 liters of 5% dextrose in one-half normal saline, cyclophosphamide in a 600-mg/m² (400 mg/m² for poor-risk patients) iv bolus, and doxorubicin in a 50-mg/m² (30 mg/m² for poor-risk patients) iv bolus; allopurinol at a dose of 300 mg orally daily was started and continued for the duration of the study. On the second day of the study, patients were given mannitol in a 12.5-g iv bolus before cisplatin at a dose of 75 mg/m² iv, followed by mannitol at a dose of 25 g in 1000 ml of 5% dextrose in water infused over a 2-hour period, and then an additional 2 liters of 5% dextrose in one-half normal saline over the next 24 hours. Additional fluids were administered to replace emesis losses. Courses were administered at 3–4-week intervals as hematologic and renal toxic effects returned to normal limits.

A complete remission (CR) was defined as the disappearance of all clinically detectable tumor. A partial remission (PR) was defined as a $\geq$ 50% reduction in measurable tumor. Minor response was defined as a reduction of measurable disease > 25% and < 50% with subjective improvement. Stable disease was defined as a response less than minor remission or increasing disease less than progression. Disease progression was defined as a > 25% increase in tumor measurement and/or the appearance of new lesion(s).

## RESULTS

Fifteen patients who had previously received no chemotherapy were entered in the study. Six of the patients had been previously treated with radiation therapy. All patients were evaluable for response and toxicity. The primary sites of carcinoma were bladder (12 patients), urethra (two), and renal pelvis (one). Evaluable metastatic sites included: bone (seven patients), lung (five), pelvic mass (four), liver (three), and lymph nodes (one). Patient ages ranged from 50 to 71 years, with a median of 63 years; there were 11 males and four females. There were 12 poor-risk and three good-risk patients, with an overall median performance status of 60% (Karnofsky scale).

PR (lymph nodes and lung) was achieved in two patients (13%), with a duration of 12 and 52 weeks and a survival of 16 and 58 weeks, respectively. Three patients (20%) showed minor response (bone and pelvic mass) and survived for 8, 28, and 102+ weeks, with subjective pain relief. Four patients' disease remained stable for 8, 18, 23, and 27 weeks. Six patients had disease progression while in the study.

As anticipated, toxicity was generally mild. Two patients experienced mild renal toxicity. Alopecia occurred to some extent in nine patients. Nausea and vomiting was severe in only four patients and was mild to moderate in six others. Five patients (33%) did not experience either nausea or vomiting. Moderate thrombocytopenia developed in three patients, while mild leukopenia developed in two and was severe in three others. One case of fatal leukopenia and thrombocytopenia was encountered in a patient who had bone marrow involvement and had received mithramycin for control of hypercalcemia. Two other patients experienced life-threatening toxicity. In general, toxicity did not appear to be cumulative.

## DISCUSSION

The development of a chemotherapeutic approach to disseminated transitional cell carcinoma is in the formative stages. While modest single-agent activity has been demonstrated for various agents, attempts to improve the quality and quantity of the responses with multiple-drug combinations have not proven clearly successful.

Previous studies of combination chemotherapy in urothelial tumors have tried to build upon the encouraging activity of cisplatin as a single agent (7,8,15).

Rossof et al (9) reported one CR and two PRs (CR plus PR, 33.3%) among nine patients with urinary bladder cancer treated with cisplatin at a dose of 75 mg/m² iv every 3 weeks. Responders had a median survival time of 30 weeks as compared to 12 weeks for nonresponders.

Williams et al (16), using the combination of cisplatin, doxorubicin, and 5-FU in 44 patients, reported that 18 patients (46.2%) achieved a PR among 39 patients evaluable for response. The median survival time for the responding patients was 10.9 months and the median survival time for the entire group was 8.5 months.

Merrin (6) reported a response rate of 37.2% (one CR and 18 PRs) in 51 patients with advanced bladder cancer treated with cisplatin at a dose of 1 mg/kg iv once weekly for the first 6 weeks and every 3 weeks thereafter. The duration of the responses varied from 2 to 13 months, with an average of 5 months.

A report from the Memorial Sloan-Kettering Cancer Center by Yagoda (8) on the use of cisplatin alone or in combination with other active agents in patients with advanced urothelial tumors showed response rates (CR plus PR) of 36%, 43%, 54%, and 50% with cisplatin alone, cisplatin and cyclophosphamide, cisplatin and doxorubicin, and CAP, re-

spectively. No significant difference in the incidence of PRs in previously untreated patients was noted.

An earlier report suggested a high response rate with the combination of CAP (17). The same three drugs, with a modification of doses because of the unacceptable toxic effects, were evaluated in patients with advanced transitional cell cancers. With modification of doses, leukopenia occurred in 53% of the patients, was life-threatening in two, and was fatal in one. Renal toxicity was minimal (13%).

In this group of previously untreated patients, two of 15 (13%) achieved a PR and 20% had a minor response. This suggested that in the above dose and schedule the combination does not appear to produce a significant response rate superior to that of single agents such as cisplatin.

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# Phase II Randomized Study of Doxorubicin, Vincristine, and 5-FU Versus Cyclophosphamide in Advanced Squamous Cell Carcinoma of the Cervix[1,2]

Brief Reports and Preliminary Communications

George A. Omura,* Enrique Vélez-García, and Robert Birch[2]

*Writing for the Southeastern Cancer Study Group*

## SUMMARY

**Sixty-one evaluable patients with advanced squamous cell carcinoma of the cervix were treated in a randomized fashion with either doxorubicin, vincristine, and 5-FU in combination (AVF) or with cyclophosphamide (CTX). Of 31 patients treated with AVF, only three (10%) responded (one complete response and two partial responses). Of 30 patients treated with CTX, there were two (7%) with partial responses. Two patients responded to AVF after failing CTX; one patient responded to CTX after failing AVF. Neither of these regimens was predictably useful in our patient population.**

[Cancer Treat Rep 65:901–903, 1981]

Although squamous cell cervical cancer can usually be cured with surgery and/or radiotherapy, a substantial minority of patients either present with very advanced disease not amenable to these modalities or have recurrences after initial treatment. There is disagreement about the value of chemotherapy in this disease. Cumulative data (1) indicated response rates ≥ 20% for single agents (cyclophosphamide [CTX], 36 of 187 patients; 5-FU, 37 of 159 patients; vincristine, ten of 34 patients; doxorubicin, nine of 28 patients; hexamethylmelamine, 11 of 40 patients; mitomycin, four of 18 patients; and methotrexate, five of 24 patients).

The present study was done to test the value of a combination of drugs (doxorubicin, vincristine, and 5-FU = AVF), all of which appeared active in cervical cancer when given alone, and which have differing toxic effects. CTX was used as a randomized control in the patient population since there was a moderately large cumulative experience with this drug, and a response rate of about 20% appeared to be established.

## MATERIALS AND METHODS

Women with histologically proven squamous cell carcinoma of the cervix whose disease was no longer amenable to curative surgery or radiotherapy and who had at least one measurable lesion were eligible, after giving written informed consent.

Patients were not eligible if (*a*) they had received prior chemotherapy with any of the agents to be used in this study, (*b*) < 1 week had passed since they had had surgery, (*c*) they were currently receiving radiotherapy, (*d*) their circumstances did not appear to permit at least 6 weeks of treatment and followup, (*e*) their absolute granulocyte count was < 1500/mm$^3$, (*f*) their platelet count was < 100,000/mm$^3$, (*g*) they had septicemia or severe infection, (*h*) there was clinically significant impairment of hepatic function (bilirubin > 1.2 mg/100 ml), or (*i*) there was renal impairment (BUN > 25 mg/100 ml or creatinine > 1.2 mg/100 ml). Patients were to have weekly blood cell counts for the first course and before each subsequent drug dose. Evaluation of response parameters was made every 3 weeks

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[2]This study was conducted by the Southeastern Cancer Study Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: University of Alabama, Birmingham (*G. A. Omura* and *R. Birch*, CA-03013, CA-19657, and CA-24456); University of Puerto Rico, San Juan (*E. Vélez-García*, CA-12223); Duke University, Durham, NC (CA-03177); Washington University, St. Louis, MO (CA-03376); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-12840); Case Western Reserve University, Cleveland, OH (CA-15584); St. Louis University, MO (CA-17214); University of Cincinnati, OH (CA-28138), University of Tennessee, Knoxville (CA-13237) and Memphis (CA-17027); Temple University, Philadelphia, PA (CA-07961), Emory University, Atlanta, GA (CA-03227), University of Louisville, KY (CA-16389), and New Jersey College of Medicine, Newark.

**Reprint requests to:* George A. Omura, MD, Department of Medicine, Division of Hematology/Oncology, School of Medicine, University of Alabama, 638 Zeigler Bldg, University Station, Birmingham, AL 35294.

except for relevant radiographs, which were repeated every 6 weeks. Eligible patients were stratified by location of lesions (pelvic vs distant sites) and by prior therapy and were then randomly assigned to receive AVF (50 mg/m$^2$ of doxorubicin by iv push on Day 1, 1.4 mg/m$^2$ of vincristine by iv push on Days 1 and 8 [maximum, 2 mg], and 500 mg/m$^2$ of 5-FU by iv push on Days 1 and 8) or CTX (1100 mg/m$^2$ by iv push on Day 1) given in courses repeated every 3 weeks. Doses (except vincristine) were reduced by 25% for the first course if prior whole pelvic radiation therapy had been given. Response was assessed after two courses; responders continued to receive the same therapy while nonresponders were crossed over to the other regimen. A complete response (CR) was defined as the disappearance of all disease. A partial response (PR) was defined as a reduction by at least 50% in the sum of the products of perpendicular diameters in all lesions. Granulocyte toxicity was rated 1+ if the count (segs + bands) decreased from normal to between 750 and 1500/mm$^3$ or 2+ if the count was < 750/mm$^3$. Platelet toxicity was rated 1+ if the count decreased from normal to 50,000–100,000/mm$^3$ and 2+ if the count was < 50,000/mm$^3$. Hematologic toxicity was rated 3+ if counts were in the 2+ range and life-threatening complications existed. Nausea and vomiting was rated 1+ if it did not require treatment, 2+ if it could be controlled by antiemetics, and 3+ if it could not be controlled. Survival curves were plotted according to the technique of Kaplan and Meier (2). The curves were statistically compared by the generalized Wilcoxon technique of Gehan (3). Response rates were compared using a chi-square test on proportions.

## RESULTS

Of 75 patients entered in the study, the data were incomplete for one, two were not eligible, three had major protocol violations, seven had inadequate trials (five were lost to followup, one refused treatment, and one had intercurrent disease preventing treatment), and one was evaluable only for toxic effects. Of these patients, eight had been randomized to receive AVF and six to receive CTX. Thus, 61 patients (31 given AVF and 30 given CTX) were evaluable for response. The age range was 31–78 years (median, 54) for the AVF group and 31–81 years (median, 51) for the CTX group.

Among patients with disease in pelvic sites only, two of the 18 given AVF had PRs but none of the nine given CTX responded. Among patients with disease in extrapelvic sites only, one of 11 given AVF had a CR and two of 13 given CTX had PRs. None of the patients with disease in mixed sites responded (two given AVF and eight given CTX). The two PRs to CTX (17 and 18 weeks) were in lung lesions, while the AVF responses were in abdominal nodes (25 weeks) and pelvic lesions (21 and 32 weeks). There were also five minor responses (25%–49% regression) to AVF but none to CTX.

Five patients (three given AVF and two given CTX) received only one course because of early disease progression. Of patients receiving two courses, one of ten given AVF had a PR but none of the 11 given CTX responded. With three or more courses, there was one CR and one PR among 18 patients given AVF and two PRs among 17 patients given CTX. All but two patients had had prior pelvic radiotherapy; of those two, one responded (PR) to AVF and the other did not. Five patients had had prior treatment with other drugs without response; none of these responded to protocol therapy. There was no significant difference in median survival between patients given AVF (30.4 weeks) and those given CTX (26.1 weeks).

Of 14 patients failing CTX and crossed over to AVF, two were not evaluable, one had a CR (4 weeks), one had a PR (24 weeks), and one had a minor response (4 weeks) to AVF. Of six AVF failures crossed over to CTX, one had a PR (4 weeks) of supraclavicular adenopathy. Reasons for not crossing over patients to the alternative treatment included poor performance status after the initial regimen (20 patients), death (three), refusal to continue (six), loss to followup (two), or no specified reason (ten). Toxicity was acceptable (table 1). There were no unexpected toxic effects; one patient receiving AVF developed congestive heart failure that was thought to be secondary to doxorubicin treatment.

## DISCUSSION

Our results with the AVF regimen were disappointing, especially since all of the study drugs in this trial have been thought to be active in cervix cancer. In particular, doxorubicin, which has a very broad spectrum of antineoplastic activity, has been reported to elicit a 32% response rate (1). Moreover, doxorubicin plus methyl-CCNU has been reported to give a 45% response rate (4) even though methyl-CCNU alone is of limited value (5). Doxorubicin plus methotrexate gave a 66% response rate (6). Nevertheless, other trials that have been published since the present study was begun would suggest that doxorubicin alone or in combination is not a consistently useful drug in cervix cancer (7–10). Cyclophosphamide was also disappointing in the present and other trials (8,10). Pelvic recurrences remain refractory to most systemic approaches; notable

TABLE 1.—Toxic effects among 31 patients given AVF and 30 patients given CTX

| Toxic effect | No. of patients with side effects after receiving— | |
|---|---|---|
| | AVF | CTX |
| Granulocytopenia | | |
| 1+ | 4 | 3 |
| 2+ | 2 | 4 |
| 3+ | 1 | 0 |
| Thrombocytopenia | | |
| 1+ | 0 | 0 |
| 2+ | 1 | 0 |
| 3+ | 0 | 0 |
| Nausea and vomiting | | |
| 1+ | 0 | 7 |
| 2+ | 1 | 2 |
| 3+ | 1* | 1 |

*1 other patient, not otherwise evaluable, had 3+ vomiting.

exceptions are the mitomycin-vincristine-bleomycin combination (11) and cisplatin (12), which appear to be active in pelvic recurrences as well as distant metastases. However, these observations need to be confirmed.

Despite a few instances in which the quality of response to AVF was impressive, our overall assessment of the present trial is that AVF was not significantly better than CTX and that both gave poor results. Controlled studies of other drugs and combinations are needed.

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# Phase II Trial of Cisplatin in Small Cell Carcinoma of the Lung[1]

Brief Reports and Preliminary Communications

Robert M. Levenson, Jr, Daniel C. Ihde,* Mark S. Huberman, Martin H. Cohen, Paul A. Bunn, and John D. Minna[2,3]

## SUMMARY

**Eighteen patients with histologically documented small cell carcinoma of the lung who had failed initial combination chemotherapy regimens were treated with single-agent cisplatin in a dose of 100 mg/m² every 3 weeks, with mannitol and fluid diuresis. Tumor regression was limited to one partial response (response rate, 6%; 95% confidence limits, 1%–27%). Significant toxic effects were gastrointestinal (severe nausea and vomiting in 12 of 14 patients) and hematologic (severe leukopenia in one patient and severe thrombocytopenia in three). The antitumor efficacy of high-dose cisplatin in heavily pretreated patients with small cell carcinoma of the lung appears to be marginal.**

[Cancer Treat Rep 65:905–907, 1981]

Cisplatin, one of the first inorganic compounds shown to possess antitumor activity against human neoplasms, is a useful drug for the treatment of testicular cancer and has demonstrated activity against bladder, ovarian, and head and neck cancers. The available data concerning the efficacy of cisplatin in small cell carcinoma of the lung (SCCL) are contradictory (1–4). This report communicates our experience with cisplatin in the treatment of patients with SCCL who had failed their primary combination chemotherapy regimens.

## MATERIALS AND METHODS

Between November 1977 and January 1980, 18 previously treated patients with a histologically proven diagnosis of SCCL received cisplatin in our unit. Patient characteristics are summarized in table 1. Eligibility criteria included normal serum creatinine level and creatinine clearance > 60 ml/minute. Patients were required to have recovered hematologically from previous chemotherapy, as shown by wbc counts > 4000/μl and platelet counts > 100,000/μl. Each patient had at least one lesion evaluable for antitumor effect. Totally bedridden patients were excluded. Most patients had pretreatment audiography.

Cisplatin was administered in an iv dose of 100 mg/m² every 3 weeks following diuresis with 12.5 g of mannitol; 19 doses were administered by 24-hour infusion, while ten were given by 1-hour infusion. Patients were hydrated for 24 hours with 5% dextrose in one-half normal saline at 125 ml/hour after drug infusion began. Responses were defined in the conventional manner. A complete response was defined as the complete disappearance of all lesions for at least 30 days, and a partial response (PR) was defined as a diminution by 50% of the sum of the products of the perpendicular diameters of measured lesions, persisting for a similar period. Progressive disease was defined as the appearance of a new lesion or a > 25% increase in the product of the perpendicular diameters of one or more existing lesions. All patients had evaluable lesions reassessed for response, except for one who died shortly after entry in the study. Cisplatin was continued until disease progression or death. Evaluable or measurable lesions consisted of chest tumors in 14 patients (including four with prior chest irradiation who had evaluable tumor at bronchoscopy or enlarging masses within or outside the radiation portal), liver metastases in three, enlarged peripheral lymph nodes in three, skin nodules in two, and bone metastases in one.

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[2]National Cancer Institute-Veterans Administration Medical Oncology Branch, Division of Cancer Treatment, National Cancer Institute, Veterans Administration Medical Center, Washington, DC.

[3]We are grateful to the many Clinical Associates who cared for these patients.

*Reprint requests to: Daniel C. Ihde, MD, National Cancer Institute-Navy Medical Oncology Branch, National Naval Medical Center, Bethesda, MD 20014.

TABLE 1.—Characteristics of 18 patients with SCCL

| Characteristic | No. of patients |
|---|---|
| Age in yrs | |
| Median: 56 | |
| Range: 45–69 | |
| Sex | |
| Males | 15 |
| Females | 3 |
| Performance status* | |
| 0–1 | 7 |
| 2–3 | 11 |
| Previous therapy | |
| Combination chemotherapy† | 18 |
| Single-agent chemotherapy | 6 |
| Irradiation (excluding cranial) | 9 |
| Disease stage | |
| Limited‡ | 6 |
| Extensive | 12 |

*ECOG-Zubrod performance status at the beginning of cisplatin therapy.

†Cyclophosphamide, methotrexate, and CCNU alternating with vincristine, doxorubicin, and procarbazine or cyclophosphamide, doxorubicin, VP-16-213, and vincristine.

‡Disease confined to 1 hemithorax, mediastinum, and ipsilateral supraclavicular nodes at the beginning of cisplatin therapy.

## RESULTS

### Antitumor Effects

No complete responses were observed. One patient experienced a brief PR (response rate, 6%; 95% confidence limits, 1%–27%) with diminution of supraclavicular and mediastinal adenopathy, but these lesions had regrown to pretreatment size by 5 weeks. This patient was unable to receive subsequent cisplatin because of thrombocytopenia and died shortly thereafter. Among the 17 nonresponding patients, progressive disease developed in six after one dose of the drug, in five after two doses, and in one after three doses. Five patients died without responding to treatment, but without documentation of progressive disease, after one or two doses. Median survival from the start of cisplatin therapy was 2 months (range, 1–13+).

### Toxic Effects

Nausea, vomiting, and myelosuppression were the most common toxic effects experienced by our patients. Twelve of 14 patients for whom detailed information is available had severe gastrointestinal toxic effects, defined as nausea and vomiting persisting for at least 12 hours. Nine patients (50%) experienced leukopenia (wbc count < 4000/μl); one episode was severe (wbc < 1000/μl). Thrombocytopenia was also common, with eight patients (44%) having platelet counts < 100,000/μl; three episodes were severe, with platelet counts < 20,000/μl. There were no episodes of serious bleeding. Three patients (16%) had levels of serum creatinine > 2.0 mg/dl; this resolved with cessation of cisplatin in two. One of three patients had a high-frequency hearing loss on followup audiogram during cisplatin therapy, though there was no clinical deterioration in hearing. There were no substantial differences in toxicity between 1- and 24-hour infusions.

## DISCUSSION

The value of cisplatin in the treatment of SCCL has been controversial, at least in part because of the variable doses employed and the intensity of prior therapy which patients had received. Several studies (1–4) of previously treated patients with SCCL have shown PR rates of 11%–36%, but these responses were generally of short duration (1–2.5 months) (2,3). Of the two trials (3,4) reporting response rates > 20%, one (4) included patients who had had no prior chemotherapy. Dombernowsky et al (2), in the largest study, found that there were only three PRs to cisplatin (11%) in 28 previously treated patients; drug doses ranged from 30 to 100 mg/m². Sierocki et al (5) observed a high response rate to cisplatin and VP-16-213 in patients with SCCL who had had no prior therapy. No further tumor regression occurred when there was subsequent treatment with cyclophosphamide, doxorubicin, and vincristine, but the contribution of cisplatin to these therapeutic results cannot be clearly delineated. Eagan et al (6) found that the addition of low-dose cisplatin to an active treatment program of VP-16-213, vincristine, cyclophosphamide, doxorubicin, and radiation therapy did not affect response rates or survival in patients with newly diagnosed limited disease.

Our disappointing experience with cisplatin in patients who had been exposed to a minimum of six drugs and were not fully ambulatory in most cases cannot be attributed to low drug dosages. We administered 100 mg/m² of the drug every 3 weeks, a dose that is intermediate between the doses given with more favorable results in two phase II SCCL studies (3,4). It is conceivable that the 24-hour infusions which we used in some patients adversely affected our results, but this is unlikely since Jacobs et al (7) found that cisplatin given by this method retained its expected activity in head and neck cancer. Myelosuppression was significant in our group of heavily pretreated patients, a problem not encountered to the same extent in other studies.

Further data will be required to determine the role of cisplatin in combination with other agents in previously untreated patients with SCCL (5,6), but its activity in patients with progressive tumor despite combination chemotherapy, and perhaps irradiation, appears to be marginal. The probable risk of increased myelosuppression in patient populations such as ours further detracts from the value of cisplatin in this setting. With the increasing intensity and number of chemotherapeutic agents which patients with SCCL presently receive, documentation of activity for all but the most active of the new phase II drugs in this disease may become quite difficult.

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# Phase II Trial of Iv 6-Thioguanine in Advanced Colorectal Carcinoma[1,2]

Brief Reports and Preliminary Communications

J. C. Britell, C. G. Moertel, L. K. Kvols, M. J. O'Connell, J. Rubin,* and A. J. Schutt[3]

## SUMMARY

**Oral 6-thioguanine (6-TG) is an S-phase antimetabolite with significant activity against acute leukemia. Preliminary reports of activity of iv 6-TG indicated significant activity against advanced colorectal cancer. We conducted a phase II study of iv 6-TG in 29 patients with advanced measurable colorectal cancer. Only one transient partial response occurred. Toxic effects included mild myelosuppression, nausea, vomiting, and minor ECG changes. We discourage further use of iv 6-TG in the dose and schedule we used to treat advanced colorectal cancer.**

[Cancer Treat Rep 65:909–910, 1981]

6-Thioguanine (6-TG) is an S-phase-specific antimetabolite which blocks purine metabolism (1). Pharmacologic studies in man have demonstrated a direct correlation between iv dose and plasma concentrations (2). The equitoxic oral dose is 2.5 times greater than the iv dose.

Although oral 6-TG is an active agent in the treatment of acute leukemia, the Eastern Cooperative Oncology Group (ECOG) was unable to demonstrate significant activity of the oral preparation in the treatment of advanced colorectal carcinoma despite significant hematologic toxicity (3). Using iv 6-TG, Denes and Presant (4) reported objective responses in three of three previously treated patients with colorectal cancer. They reported no cumulative myelosuppression but have noted minor cardiac changes. To further evaluate the activity of this preparation, we performed a phase II study of iv 6-TG in patients with advanced colorectal cancer.

## MATERIALS AND METHODS

Twenty-nine patients with histologically proven and measurable metastatic or residual colorectal carcinoma were entered in this study between October 1979 and March 1980. Fifteen patients had received prior chemotherapy with a variety of agents including 5-FU (12 patients), PALA (seven), methyl-CCNU (four), chlorozotocin (three), indicine-*N*-oxide (three), ftorafur (one), mitomycin (one), triazinate (one), and methyl-GAG (one). These patients had a median age of 66 years. Fourteen patients had received no prior chemotherapy. Their median age was 65 years. Nineteen patients had ECOG performance scores of 0–1 and ten had performance scores of 2–3. Sites of metastases included liver (24 patients), abdomen (four), and lung (four).

All patients had pretreatment wbc counts > 4100/mm$^3$, platelet counts > 130,000/mm$^3$, and serum creatinine < 2.0 mg/100 ml.

Previously treated patients received 6-TG at a dose of 700 mg/m$^2$ iv every 21 days. In the absence of progressive disease, myelosuppression, or other severe toxicity, the dose was escalated to 1000 mg/m$^2$ for the second course of therapy and by 20% for every course thereafter. Patients without prior therapy began 6-TG at a dose of 1000 mg/m$^2$.

Initially, the 6-TG was prepared by diluting a 75-mg drug vial with 5 ml of normal saline. Infusion time was expected to be 10 minutes. Venospasm and pain in the first three patients prevented administration in < 1 hour. Further dilution of the drug with an additional 5 ml of normal saline and 0.5 ml of $NaHCO_3$/75-mg drug vial and infusion over 30 minutes prevented these complications.

Patients were re-evaluated at the end of each 3-week treatment cycle. Response criteria were defined as a > 50% decrease in the product of perpendicular diameters of the primary indicator lesion

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[3]Mayo Clinic and Mayo Foundation, Rochester, MN.

*Reprint requests to:* Joseph Rubin, MD, Medical Oncology and Internal Medicine, Mayo Clinic and Mayo Foundation, Rochester, MN 55901.

or a 30% decrease in the sum of the distances below the xiphoid process and costal margins if malignant hepatomegaly was the indicator lesion. Stable disease was defined as a < 50% reduction or a < 25% increase in the product of the perpendicular diameters of the indicator lesion, or a < 30% reduction or a < 25% increase in the sum of the distances below the costal margins and xiphoid process for hepatomegaly. Disease progression was defined as a > 25% increase in the product of the perpendicular diameters of the indicator lesions or in the sum of the distances below the costal margins and xiphoid process for malignant hepatomegaly, development of new lesions, deterioration in performance score (> 1 level of ECOG scale), or weight loss ≥ 10% of initial body weight.

## RESULTS

Only one of 29 patients achieved a partial regression. She was an 80-year-old female with no previous therapy. After two cycles of therapy her abdominal mass decreased by 60%. Although she had mild subjective improvement after her first cycle of 6-TG, at the time her regression was noted she was complaining of increasing pain. The duration of her regression was 15 weeks. Twelve of 29 patients remained objectively stable 3–12 weeks after initiation of therapy. Sixteen of 29 patients showed progressive disease within 3 weeks.

### Toxicity

Problems with local infusion, venospasm, pain, and erythema over the vein occurred only in the first three patients treated prior to the addition of $NaHCO_3$ and extra normal saline to the drug preparation. Moderate to severe nausea occurred in 15 of 29 patients (52%), and vomiting of moderate or severe degree occurred in 11 (35%). Mild stomatitis occurred in one patient and moderate or severe diarrhea occurred in three.

Myelosuppression (wbcs < 4000/mm³; range, 3100–3900) occurred in five patients treated at a dose of 700 mg/m². One patient developed thrombocytopenia (platelets, 75,000/mm³) at this dose. Of 17 patients treated at a dose of 1000 mg/m², five developed leukopenia during the first cycle of therapy (range, 2000–3800 cells/mm³). Thrombocytopenia (platelets, 89,000/mm³) occurred in only one patient treated at a dose of 1000 mg/m². Of the five patients with first-cycle leukopenia, two demonstrated cumulative myelosuppression, with wbc count nadirs of 1500 and 500/mm³ on subsequent cycles.

Cardiac toxicity was monitored by obtaining 12 lead ECGs immediately prior to treatment, at the completion of drug infusion, at 3 hours following infusion, at any time during this period if the patient complained of headaches, palpitations, or dizziness, and at 24 hours after treatment. At a 6-TG dose of 700 mg/m², two of 13 patients developed bradycardia and nonspecific T-wave flattening, which resolved within 3 hours. At a dose of 1000 mg/m², five of 19 patients demonstrated minor changes: bradycardia (two patients), nonspecific ST- and T-wave changes (five), or P-wave changes (one). The only patient treated at a dose of 1200 mg/m² developed nonspecific ST-T-wave changes.

## CONCLUSIONS

Only one very transient partial regression was observed among 29 patients with advanced colorectal cancer. In view of the minimal demonstrated activity against advanced colorectal adenocarcinoma, further use of iv 6-TG for this type of disease, at the dose and schedules we used, should be discouraged.

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# Summary of the Workshop on Intraoperative Radiation Therapy[1]

**Joel Tepper* and William Sindelar[2]**

Radiation therapy delivered during a surgical procedure has the advantage of allowing the radiation therapist to remove the normal tissues from the radiation field while having a well-defined tumor volume to irradiate, thereby allowing a higher dose of radiation to be delivered to the tumor while relatively sparing normal tissues. Although the potential advantages of the technique have been realized for almost 50 years, technical limitations have impeded the development of intraoperative radiation therapy. These limitations have included a lack of higher beam energies and surgical support. Over the last 10 years in Japan and the last 3 years in the US (table 1), there has been a resurgent interest in intraoperative radiation therapy. A workshop was held in March 1980 to review the present status of intraoperative radiation therapy in the US and to help outline areas of interest for future research. The following is a summary of the issues and experiences discussed by representatives of the various institutions involved.

## CLINICAL EXPERIENCE

### Massachusetts General Hospital (L. Gunderson and W. Shipley)

The approach at the MGH has been conventional external-beam radiation therapy in combination with intraoperative electron-beam radiation therapy, using the intraoperative field as a boost dose to help control the regions of bulk disease. This approach is consistent with the philosophy used in radiation therapy for other sites where a boost dose is often delivered with external beam or by interstitial or intracavitary implantation. The criteria for treatment with intraoperative radiation therapy at the MGH are: ability of the patient to tolerate surgery; treatment plan where the patient would have otherwise received external-beam radiation therapy with curative intent to a dose of 6000–7000 rad; and likelihood that intraoperative radiation therapy, because of the ability to spare normal tissue, would improve the therapeutic ratio.

Patients were treated from early 1978 through December 1979, with an analysis performed in January 1980. During that time, 56 patients were evaluated and 36 were eventually treated. Fifty-two patients had lesions in the abdomen or the pelvis and four patients had soft tissue sarcomas of the extremities. Forty-four of the 56 patients had gastrointestinal primaries (26 were rectal, 16 were pancreatic, and two were gastric in origin). Five

TABLE 1.—Experience with intraoperative radiotherapy in the US (1980)

| Institution | No. of patients | Sites of diseases treated | No. of protocol studies |
|---|---|---|---|
| Howard University, Washington, DC | 43 | Lung<br>Breast<br>Esophagus<br>Stomach<br>Pancreas<br>Cervix<br>Colon | 0 |
| Massachusetts General Hospital (MGH), Boston | 36 | Stomach<br>Pancreas<br>Sarcomas | 0 |
| National Cancer Institute (NCI), Bethesda, MD | 6 | Stomach<br>Pancreas<br>Sarcomas | 3 |

[1]Workshop on Intraoperative Radiation Therapy, Bethesda, MD, March 14, 1980. Participants included J. Fowler, E. Glatstein, D. Pistenma, W. Sindelar, J. Tepper, and E. Travis (National Cancer Institute, Bethesda, MD); P. Biggs, L. Gunderson, and W. Shipley (Massachusetts General Hospital, Boston); E. Cornwell, A. Goldson, R. Nibhanupudy, L. Rayford, and V. Roux (Howard University, Washington, DC); J. Chaffey, K. Kase, A. Malcolm, and T. Rich (Joint Center for Radiation Therapy, Boston, MA); K. Drake and H. Hoover (Johns Hopkins Hospital, Baltimore, MD); and E. Gillette (Colorado State University, Fort Collins).

[2]Radiation Oncology Branch (*J. Tepper*) and Surgery Branch (*W. Sindelar*), Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Dr. Joel Tepper, Department of Radiation Medicine, Massachusetts General Hospital, Boston, MA 02114.

gynecologic tumors were also evaluated. Of the 36 treated patients, breakdown by tumor was: 26 gastrointestinal tumors (17 colorectal, eight pancreatic, and one gastric), four gynecologic tumors, and six sarcomas (two pelvic and four of the extremities). Of the 20 evaluated patients who were not treated, nine had evidence of disease outside of the local area or the patient refused intraoperative radiation therapy; for the remaining 11 patients, the decision not to receive intraoperative radiation was made for a variety of medical and logistic reasons. A total of 47 patients were taken to surgery with the consideration of intraoperative radiotherapy.

A further breakdown of the 36 patients who were treated showed that 12 had tumors classified as recurrent and unresectable, 17 had unresectable primary tumors, five had primary tumors with residual disease after surgical resection, and two had primary disease which was resected but with a very high risk of local recurrence.

There are significant technical problems involved in performing intraoperative radiation therapy. Specificially, all surgery is performed in the operating room suite, and then the patient is transported, while still under general anesthesia, to the radiation therapy department. Obviously, there must be excellent coordination and a significant amount of effort expended by many people for the procedure to run smoothly. Sixteen surgeons were involved with the 47 patients who went to operation with consideration of intraoperative radiation therapy. Patients were treated with electron energies ranging from 6 to 29 mev. Nine of the 36 patients were treated with electron energies of $> 18$ mev. The external-beam dose generally was 4500–5000 rad and was delivered preoperatively except in the patients with pancreatic cancer. In this latter group, there was a desire to have the option of an implant as well as the intraoperative radiation therapy, so only 1000–2000 rad was given preoperatively and the rest was given after surgery. The intraoperative radiation therapy dose was 1000–1500 rad in 33 of the 36 patients. Because of two local recurrences in the patients with pancreatic cancer, the dose for this tumor has recently been increased to 1750 rad.

Because of the problems with transporting the patient into an area not generally used for surgical procedures, there has been concern about the possibility of postoperative infection. There were four infections among the 36 irradiated patients. In all these cases, it was likely that the nature of the surgical procedure, rather than the transportation or radiation procedures, predisposed to infectious complications.

Thirty-five of the 36 patients were considered evaluable at the time of the workshop. One patient died from rupture of an iliac vessel 1–2 months after the completion of treatment. It is uncertain whether this rupture was related to the tumor or to the treatment. Four of the 35 evaluable patients have had evidence of local tumor progression. For 20 of the 29 patients who presented with unresectable disease, the initial therapeutic intent was to use preoperative irradiation to make the disease surgically resectable. This was accomplished in 14 of 20 patients. Six of 36 patients died, and another four were alive with disease. It is of interest that all of the local recurrences occurred in patients where gross tumor was irradiated, in whom resections could not be or were not performed. It is too early to make any comments with regard to cure rates, but the complication rate at present seems to be quite acceptable and there appears to be good local disease control with short followup.

There are special surgical considerations in approaching a patient who will receive intraoperative radiation therapy. The further cephalad the location of the tumor in the upper abdomen, the greater the technical problem produced by the rib cage in delivering the intraoperative radiation therapy. Thus, the surgical incision must be designed to allow good access to this region, always allowing generous exposure. Two patients with carcinoma of the pancreas who were treated early in the series had a small portion of the stomach irradiated. One of these patients developed a prepyloric obstruction secondary to the radiation therapy. This is the only patient in the series who had not had a routine gastroenterostomy, but the obstruction was subsequently relieved with a bypass procedure. It is thus recommended that a routine gastroenterostomy bypass procedure be performed in all patients with carcinoma of the pancreas. The second patient in whom a portion of stomach was irradiated developed gastric bleeding and was found to have ulceration in the prepyloric region. This was subsequently controlled by conservative treatment. A small portion of the duodenum was irradiated in all patients with carcinoma of the pancreas left in situ, but these patients have not developed significant problems. Different difficulties arise when treating lesions in the pelvis. In all patients, it was possible to remove small bowel from the field. Ureters are considered to be potentially dose-limiting structures. The rectum was irradiated in two patients, without complications. The ureter was in the field of irradiation in three patients. One patient developed ureteral obstruction and has required a stent postoperatively. A portion of the bladder was irradiated in three patients; all developed temporary incontinence which resolved spontaneously. When treating lesions in the pelvis, it is often necessary to use a

very steep angulation of the machine gantry, or to use beveled cones to obtain good access to the tumor volume. In certain situations it was also found beneficial to use a perineal approach. Certainly, further technical advances must be made to improve the ability to treat the relatively inaccessible regions.

### Howard University (A. Goldson and E. Cornwell)

Howard University began intraoperative radiation therapy in 1976. Most patients treated have had far-advanced disease and have been treated to establish the maximum dose which would be tolerated by normal structures. The dose therefore has ranged from 1000 to 3000 rad in a single fraction. The emphasis most recently has been on pancreatic carcinoma and on periaortic lymph node irradiation. Howard University has converted a standard radiation therapy treatment room into a fully equipped operating room. The treatment couch has been removed and has been replaced with a standard operating room table. This entirely avoids the problem of patient transportation. The radiation therapy department is adjacent to the main operating room, so full surgical support facilities are easily available.

Forty-three patients have been treated (20 males and 23 females). A large percentage of them have had unresectable tumors. Seventeen patients had pancreatic primaries, 12 patients had periaortic lymph node irradiation, and the remaining patients had a variety of primaries including carcinomas of the lung, biliary tract, stomach, breast, colon, and bladder. A few patients had mediastinal irradiation, but because of esophageal complications at a dose of 2000 rad, treatments in that area have been discontinued. Patients are treated through circular cones which vary from 6 to 9.6 cm in diameter, except for the periaortic lymph node irradiation where rectangular cones measuring 6 × 10 or 6 × 15 cm are used. Electron energies have varied from 9 to 18 mev, with a dose rate of 500 rad/minute. Twelve of the 17 patients with pancreatic carcinoma had liver metastases and were treated as part of a phase I study. In this group, it was demonstrated that jaundiced patients who had preoperative decompression of the biliary system have tolerated the surgical procedure better than those who did not have preoperative decompression. Many had the decompression percutaneously, and this is now standard procedure. Hyperalimentation is frequently used preoperatively. Usual surgical exposure is through a bilateral subcostal incision which allows good visualization of the tumor and adjacent intra-abdominal contents. Pancreatic carcinomas have not been resected in any patients, and very few patients, to date, have received any additional external-beam radiation therapy. Only two of the patients with pancreatic carcinoma lived > 6 months; the longest survival was 12 months. There was no 30-day mortality and no significant problems with wound infection. There is some suggestion from this experience that more complications may occur with higher doses and with larger treatment volumes. Howard University's future plans are to combine intraoperative irradiation with postoperative external-beam radiotherapy to a dose of 4000–5000 rad, in an attempt to improve survival.

### National Cancer Institute (J. Tepper and W. Sindelar)

The treatment plan at the NCI with intraoperative radiation therapy has philosophically differed somewhat from the approaches at Howard University and the Massachusetts General Hospital. The approach has been to use intraoperative radiation therapy as a postoperative adjunct after a radical surgical resection of the tumor, with additional external-beam radiation therapy to be used only in selected clinical situations. In addition, after the first few patients, all patients have been treated with the hypoxic cell radiosensitizer misonidazole since it is thought that when large single doses of radiation are being delivered, hypoxic cells may be the dose-limiting tissue for tumor control. Misonidazole is given iv approximately 15–30 minutes before irradiation. The initial dose of the drug was 1.5 g/m$^2$ but has subsequently been increased to 3.5 g/m$^2$.

At the NCI, the patient must be transported from the operating room to the radiation therapy area. Despite significant potential logistic problems, the transportation has proceeded without difficulty in all cases. During the radiation therapy, patients are monitored by closed-circuit television. Outside the treatment room, there is constant monitoring of ECG, blood pressure (obtained with an arterial line), and respiration. There have been no anesthesia problems during intraoperative radiation therapy. The dose of intraoperative radiation therapy has been 2000 rad, and radiation therapy has been delivered with 11-mev electrons with the dose quoted at the 90% isodose line.

Nine patients have been considered appropriate for intraoperative radiation therapy. Three patients were not irradiated: two had metastatic disease, and one who had lost approximately 30 units of blood during extensive retroperitoneal tumor resection was considered too clinically unstable for the transpor-

tation procedure. Three patients have had secondary lead shielding which is done with a custom cutout at the time of the surgical procedure in order to protect additional normal tissue. The shield is cut at the operating table from presterilized lead sheets.

There are a few specific observations which are of interest with regard to some of these patients. There have been no complications which can be attributed directly to the intraoperative radiation therapy. One patient had a resection and re-anastomosis of a portal vein which was involved with a pancreatic adenocarcinoma. A regional pancreatectomy was performed which included a resection of the portal vein. This anastomotic site was irradiated intraoperatively without any postoperative difficulties. Another patient had irradiation of a closed duodenal stump to a dose of 1200 rad following gastrectomy and partial hepatectomy and showed no evidence of subsequent leakage from the irradiated duodenum. One patient had a portion of the rectum irradiated intraoperatively, as this was closely adherent to the tumor bed. The patient developed diarrhea which lasted for 1 month after surgery and stopped spontaneously. Two patients had a portion of the biliary system irradiated and postoperatively developed a transient functional biliary obstruction. This obstruction was characterized by a modest rise in the liver enzymes and bilirubin, lasted approximately 1 week after surgery, and resolved spontaneously.

The incision is of critical importance both for adequacy of the surgical procedure and for adequacy of exposure for the radiation therapy. It has been found that for upper abdominal lesions, a bilateral subcostal incision provides excellent exposure. For the pancreatic resections an upper midline incision also gives adequate access to the celiac axis and superior mesenteric vessels but may make radiation of the porta hepatis somewhat difficult in patients with a narrow costosternal angle. The radiation therapy is delivered after tumor resection, but before any gastrointestinal anastomoses are performed. Irradiation prior to restoring gastrointestinal continuity allows wide exposure of the resection bed and avoids irradiating normal portions of the intestinal tract. Also of interest is the observation that patients who have major surgical resections in combination with the intraoperative radiation therapy have a very large third-space fluid requirement during the postoperative period. Two patients with extensive retroperitoneal dissections have developed ascites postoperatively and seem very much like they have had a retroperitoneal burn. A rather low-grade nonspiking fever is also present for about 1 week after surgery. These observations have not been reported previously.

There are presently three prospective randomized protocols which have been activated to analyze the value of intraoperative radiation therapy. Because of the constraints of coordinating and planning intraoperative radiation therapy, the randomization is performed prior to surgery and, if the patients at surgery are found to have previously unrecognized metastatic disease or disease that is deemed entirely unresectable, they are removed from study. The first protocol is for patients with adenocarcinoma of the pancreas; patients are divided into one of three groups. Group 1 contains patients whose disease would be considered resectable by conventional criteria. These patients have negative microscopic margins, no lymph nodes involved with tumor, and no evidence of invasion into adjacent soft tissue. They are randomized to receive surgery with intraoperative radiation therapy (2000 rad) plus 3.5 $g/m^2$ of iv misonidazole versus surgical resection alone. It is expected that there will be few patients who fit these ideal criteria of tumor resectability. Group 2 contains patients who have marginally resectable disease. These patients have positive microscopic resection margins or positive lymph node disease. They may have disease adherent to the portal vein, superior mesenteric vessels, or other structures, but they have no gross residual disease remaining at the end of the surgical procedure. Their tumor would be considered unresectable at most institutions. These patients are randomized to receive surgery plus intraoperative radiation therapy and misonidazole versus surgery plus conventional postoperative irradiation therapy to a dose of 4500 rad. Group 3 contains patients for whom it is thought at the beginning of the surgical procedure that a gross resection of the tumor can be obtained, but with gross residual disease remaining after surgery is completed. The treatment in this group of patients is the same as that in group 2, but group 3 is analyzed separately because of the gross residual tumor. There is a protocol for carcinoma of the stomach which is designed similarly to the pancreatic adenocarcinoma study. The group classification definitions include patients in group 1 who have no nodal involvement and no involvement through the serosal wall of the stomach, patients in group 2 who have serosal involvement, positive microscopic margins, or positive lymph nodes, and patients in group 3 who have gross residual disease after resection. The third protocol which has been activated is for patients with retroperitoneal soft tissue sarcomas. The impetus behind this study is that at the NCI, there has been an ongoing evaluation of treatment of retroperitoneal soft tissue sarcomas with maximal surgical resection and postoperative resection. In the patients who have been treated to date with

conventional radiation therapy, the local control has been extremely good (15 of 17 patients) but the morbidity has been high, including two treatment-related deaths. It is hoped that intraoperative radiation therapy will reduce the treatment-related morbidity and mortality. Patients in this protocol all receive maximal surgical resection and receive, in randomized fashion, either intraoperative radiation therapy plus external-beam irradiation to a dose of 3500–4500 rad or conventional postoperative irradiation to 5000 rad. There is then a second randomization analyzing the value of adjuvant chemotherapy in the same group of patients.

It is extremely important that terms are used in a consistent manner. For instance, the definition of "resectable" needs to be well-defined among the different groups involved in using intraoperative radiation therapy. Most of the patients with pancreatic carcinoma resected at the NCI would be considered to have "unresectable" tumor by most surgeons, as would many of the patients with gastric cancers and retroperitoneal sarcomas. The operating definition of resectability has been the responsible surgeon's belief that all gross tumor can be removed. It is worth noting that all patients are evaluated very carefully for evidence of liver metastases or peritoneal seeding. Thus, it is felt that randomized trials in all of these groups are essential in order to evaluate the situation properly.

## TECHNICAL APPROACHES

### National Cancer Institute (T. Padikal and J. Tepper)

The electron-beam facilities at the NCI are presently limited to a maximum electron-beam energy of 11 mev which gives a maximum electron range of 3.5 cm. There are firm plans to proceed with a room in a new radiation therapy department, currently under construction, which will be designed to accommodate a complete operating room including all ancillary support equipment. In designing a new treatment area and ordering a new treatment machine, there are a number of criteria which must be evaluated with regard to the room, the machine, the cone, and the beam characteristics. A room in the radiation therapy department must be appropriately isolated from the rest of the department so that it can function as a "clean" area for an operating suite, but it must be versatile enough to be used for conventional radiation therapy when surgery is not scheduled. The machine must be positioned in such a way that there is adequate space for the standard operating procedures as well as for the intraoperative radiation therapy and that rapid room entry and easy cleaning can be accomplished. The machine hardware must also be versatile enough so that there are well-defined slow-motion movements of the table. A gantry-mounted camera is extremely helpful in allowing visualization of the radiation field. There should be a transparent cover over the treatment cone to prevent any dust or other material from falling from the machine into the open wound. There must be an adequate number of degrees of freedom on the machine so that one can obtain various angulations of the gantry with respect to the patient. Last, there should be a rapid-disconnect table so that both a radiation therapy treatment couch and conventional operating room table can be employed, or the radiation therapy table must be modified so that it is usable as an operating room table.

In electron-radiation therapy, cones are standardly used to define the radiation fields, but in intraoperative radiation therapy they also act as retractors to hold normal tissue out of the field. These cones must be of various sizes and shapes so as to conform to various anatomic situations in the patient. They must be mechanically rigid so they can function as a normal tissue retractor. It is important that they be transparent so that the radiation therapist can visualize through the cone to confirm that the proper tissues are being irradiated. There must be a rapid and easy-disconnect system so that they can be removed without difficulty. Obviously, they must be made of a material that can be sterilized and they should be designed so that they will not shatter if subjected to stress. It would be helpful if there were pressure sensors on the tips of the cones so that excess pressure is not placed on intraabdominal structures in the patient. It would also be useful to have slots on the cones through which one could attach a viewing device to look along the beam axis, insert measuring devices and illumination devices, or position suction tubing to clear blood and serum that tend to accumulate during treatment.

There are obviously many different treatment machines which can be used for intraoperative radiation therapy. There are a number of different criteria that an intraoperative radiation therapy machine ideally should meet. There should be maximum radiation dose on the surface, as the tumor is usually present directly on the surface (this is contrary to many of the other uses of electron beams where some sparing of superficial tissue is beneficial). The dose then should be as uniform as possible across the tumor with a rapid fall-off in dose past the distal end of the tumor. The total beam penetration should be variable, with a maximum pene-

tration to as large a depth as possible (5–7 cm being highly desirable). There should be minimal X-ray contamination of the beam. Because of the large doses given in a single treatment, a high dose rate of the order of 1000 rad/minute is quite desirable. Obviously, as for all radiation therapy machines, much attention must be paid to details of the dosimetry, with redundant monitoring systems and safety checks. These criteria probably are achievable with present technology, and one should try to achieve them if at all possible.

### Howard University (R. Nibhanupudy and A. Goldson)

The physical setup at Howard University employs an operating room in the present radiation therapy department. Presently, electrons are available with energies of 6, 9, 12, 15, and 18 mev. Patients are viewed from behind a leaded glass window in a lead-lined door. Although there have been no problems in irradiating the patient with the anesthesia team outside of the room, consideration is being given to using a lead shield which can be placed inside the treatment room and close to the patient. The anesthesiologist would be able to turn off the machine immediately if the need arose. Preliminary calculations show that for a 6-mev electron beam an observer in the room would receive 4 millirads for each 2000-rad dose to the patient. For 18-mev electrons, the dose behind this lead booth would be approximately 9 millirads. To attain these doses, a 1/16-inch lead shield approximately 8 feet long and 3 feet wide would be needed. More work will need to be done to determine if this is a feasible approach.

### Massachusetts General Hospital (P. Biggs)

The size of the radiation therapy room in the MGH is approximately 25 × 25 feet. The room is used only for radiation, as the surgery is performed in the regular operating room. The collimation system from a Cobalt-60 unit has been placed into the head of the Clinac 35 in order to obtain a better dosimetry system. Higher energies are available on this machine than on machines at Howard University or at the NCI, and electron energies of up to 29 mev have been used. Eleven of 39 patients have received an energy > 18 mev. The most common cone diameter size is between 6 and 7 cm. A few cones are available with a beveled edge in order to obtain good access into regions deep into the pelvis when angled-gantry docking approaches are used. For the pelvic region, it has been found that a wall thickness on the cone of ½ inch is too great because of the difficulties with a tight fit. The surface dose was investigated, and it was found that for 15-mev electrons the surface dose is 90% of the maximum dose, but for 6-mev electron energies it decreases to 82% or 83%. Thus, for all but the lowest energies the surface dose is quite close to the prescribed dose, which is 90% of the maximum dose. Details of the collimation system and of the scattering foil system are critical in order to optimize the dose distribution of the patient. Improper scattering foils or improper collimation settings can result in a greater degree of infield beam contamination which can increase the dose to distal normal structures. It can also result in a more gradual fall off of the electron dose distally, also resulting in undesirable irradiation of distal structures.

### Joint Center for Radiation Therapy (K. Kase and J. Chaffey)

The present plans at the Joint Center for Radiation Therapy (JCRT) is to use an orthovoltage machine to irradiate microscopic residual disease after gross resection of tumors in the upper abdomen, pelvis, and perhaps the chest. There are three possibilities for location of a treatment machine for intraoperative radiation therapy: to have a dedicated room and a dedicated machine for intraoperative radiation therapy and surgery, to have the treatment machine in the radiation therapy department and transport the patient from the operating room to the treatment area, or to have a treatment machine adjacent to an operating room so that patients may be accessed from several other operating rooms after transporting them a minimal distance. The latter situation has the advantage that there may be at any single time many patients available in the surgery suite for potential intraoperative radiation therapy. Present plans are to use a Phillips orthovoltage machine with half-value layer of up to 7 mm of copper. The machine is presently available at the JCRT and the cost estimate for outfitting the room and transporting the machine is modest. Plans are to mount the machine on ceiling tracks to make it quite mobile. Standard-design orthovoltage treatment cones will also be employed. For this machine, with projected filtration, the 50% isodose line is approximately 5–6 cm deep in tissue. For a typical case of pancreatic carcinoma it is estimated that the spinal cord dose would be approximately 25%–30%. There may be a significant limitation because of a maximum dose rate, which is projected to vary between 225 and 330 rad/minute depending on the focal spot to skin distance and the filtration. Be-

cause of the low energy of the beam, there is increased dose absorption in bone as compared to muscle by a factor of approximately 1.4. There is, however, a significant difference in absorption characteristics between the value calculated from mean energy of an orthovoltage beam and the measured value. There are some situations where the increased absorption in bone combined with the increased biologic effectiveness of orthovoltage might make treatment with this machine somewhat difficult. This would also be affected by the total dose to be given through the intraoperative field. However, the low cost of using an orthovoltage unit which is presently available in the department makes this technique of intraoperative radiation therapy potentially very appealing.

## RADIOBIOLOGY

### National Cancer Institute (W. Sindelar)

The NCI began investigating the radiobiologic aspects of intraoperative radiation therapy in late 1978. Three basic questions were initially asked: how much radiation could be delivered to normal structures, what sort of complications might develop in normal structures, and what types of surgical procedures could be performed in the face of a dose of intraoperative radiation therapy? Dogs were irradiated with an 11-mev electron beam from the Armed Forces Radiobiologic Research Institute (AFRRI). Attempts were made to duplicate the clinical situation of intraoperative radiation therapy as closely as possible. Irradiations were performed through a 3½ × 12-cm cone which covered the retroperitoneal structures and extended above the renal arteries to the level of the aortic bifurcation. A full dose was delivered to the aorta and inferior vena cava, and the kidneys were intended to be out of the radiation field. The abdominal viscera were also removed from the radiation field. Because the AFRRI accelerator has a fixed horizontal beam, the animal had to be turned to its side, left side up, so that it could be docked to the accelerator. Dogs were irradiated at doses of 2000, 3000, 4000, and 5000 rad. In a second experiment, dogs were irradiated after an aortic trans-section and re-anastomosis. A third experiment involved irradiating a blind loop of duodenum. In these experiments, dogs with anastomoses were irradiated at doses of 2000, 3000, and 4500 rad. Over 40 animals have been used in various experiments. Although the data presently available are preliminary, the irradiations were generally tolerated very well. A few of the dogs who had a blind loop of duodenum irradiated developed an intussusception approximately 5–7 days after the irradiation, requiring surgical correction. A few dogs who had ureter in the field at higher doses (> 3000 rad) developed stenotic changes in the ureter. In a few dogs a left kidney was inadvertently irradiated, and this resulted in almost total obliteration of the normal architecture of the kidney, with nonfunctioning of that kidney. One animal also had a portion of the rectum inadvertently irradiated, and a rectal perforation developed at approximately 2 months (4000 rad). The aorta, vena cava, and retroperitoneum consistently showed minimal changes for time periods as long as 1 year after irradiation. Mild fibrotic changes were seen in the aortic anastomoses at the late followup times, with one fistula developing between the aorta and vena cava at the anastomotic site.

In summary, there appeared to be little difficulty in tolerating doses of 3000 rad, there was some evidence of normal tissue complications at 4000 rad, specifically in the ureter, and there may be greater problems, especially in anastomotic sites, at doses of 4500 rad. These animals will be followed for longer time periods, but these preliminary results are encouraging, and it appears that the doses of 2000–3000 rad which are being used clinically at the National Cancer Institute, Massachusetts General Hospital, and Howard University should be well-tolerated.

### Howard University (A. Goldson)

A series of animal experiments had been performed earlier at Howard University irradiating a segment of the aorta and vena cava to doses of 3000 and 4000 rad. At 16 months there were no major problems in either of these great vessels. This is consistent with the National Cancer Institute data. The most recent radiobiology experiments at Howard University have been performed on dogs. Two structures were irradiated, a 10-cm length of duodenum and the whole bladder. The duodenum developed some contraction. At 1 week it was quite hemorrhagic with marked edema, and at 8 weeks it was often necrotic with ulceration. This effect was more pronounced at the higher radiation dose of 4000 rad than at 3000 rad. The bladder, which was also irradiated in its entirety, showed significant contraction and hydroureter at 8 weeks, especially at the 4000-rad dose level. Most of the animals had a significant weight loss and died at relatively early times.

### Colorado State University (E. Gillette)

In a preliminary study in dogs, a 10-cm segment of colon was irradiated. Within 4 months, all of the

animals died from the irradiation-induced marked colonic structure, even with doses as low as 1500 rad. A design is being developed for future radiobiology experiments to look at the tolerance of normal structures. Three treatment arms are planned: (*a*) intraoperative radiation therapy alone to doses of 2000, 2500, 3000, and 3500 rad; (*b*) 5000-rad external-beam radiation therapy given in divided fractions with a boost dose of intraoperative radiation therapy of doses 1000, 1500, 2000, and 2500 rad; and (*c*) external radiation therapy alone of 5000, 6000, and 7000 rad given in divided doses. It is hoped that these treatment schemes will allow some determination of the relative biologic effect of the dose presently being used in the intraoperative irradiation programs. As there is reason to believe that the microvasculature may be the dose-limiting structure for long-term damage to normal structures, a careful investigation of the microcirculation is planned. Also, it would be of significant interest to analyze the effect of hypoxic cell sensitizers or normal tissue radioprotectors combined with intraoperative radiation therapy. For these experiments, it would be very helpful if experiments could be designed using spontaneous tumors in dogs. Preliminary experiments performed with the radioprotector WR2721 indicate virtually no concentration of this agent in animal tumors. The potential advantage of using a hypoxic cell radiosensitizer such as misonidazole seems to be great in intraoperative radiation therapy. At the iv dose of 3.5 g/m$^2$, one would expect a serum concentration of 100 μg/ml which should give a dose-reduction factor of approximately 1.7 for hypoxic cells. This should be as good as one could expect from heavy charged-particle irradiation.

## CONFERENCE SUMMARY (D. Pistenma, NCI)

Much valuable information on different approaches to intraoperative radiation therapy has been transmitted at this workshop. Because this approach may well make a significant improvement in the local control of various tumors, it is extremely important that there be a strong effort to standardize guidelines for describing the various parameters related to intraoperative radiation therapy, specifically that the extent of local tumor, the tumor dose, and the type of surgical resections performed be described consistently and in a uniform manner. This will go a long way to define more accurately the problems involved with intraoperative radiation therapy and to promote significant advances.

## *Allergic Reaction Following Administration of AMSA*[1]

AMSA is a new acridine derivative with important antileukemic activity (1,2). Its side effects include nausea, vomiting, stomatitis, alopecia, pancytopenia, hepatic dysfunction, and, rarely, cardiac arrhythmias and seizures. Here we describe the occurrence of an acute skin reaction following administration of AMSA and the response to pretreatment with Benadryl and/or hydrocortisone.

### CASE REPORT

A 32-year-old white male had a wbc count of 100,000/mm$^3$ during a routine examination in September 1979. A diagnosis of chronic myelocytic leukemia was made, with demonstration of the Philadelphia chromosome. He was treated with hydroxyurea until July 1980 when he showed weight loss and anemia. A repeat bone marrow aspiration confirmed blastic transformation. He was treated with vincristine and prednisone without response. On September 27, 1980, he was admitted to Memorial Hospital for further chemotherapy. Physical examination at admission was significant for normal vital signs. His skin was clear and his spleen was enlarged. His blood tests included a wbc count of 23.8/mm$^3$, a hemoglobin of 9.9 g/100 ml, and a platelet count of 215,000/mm$^3$. SMA-12, prothrombin time, partial thromboplastin time, chest roentgenogram, ECG, and urinalysis were normal. He started receiving AMSA, cytarabine, and 6-thioguanine (3) on September 29, 1980. Treatment began with a 1-hour infusion of AMSA at a dose of 240 mg in 250 ml of 5% dextrose in water. Soon after the infusion started, the patient noted nasal stuffiness. He then developed a pruritic, erythematous, blanching, nonurticarial rash over his chest, upper back, and neck. The rash thereafter generalized. His vital signs remained stable, his chest remained clear without wheezing, and he denied respiratory difficulties. He was treated with iv Benadryl at a dose of 25 mg and the rash disappeared when the infusion was completed. He then started receiving cytarabine (bolus and infusion) and 6-thioguanine (orally). The next day he was premedicated with 25 mg of Benadryl prior to administration of AMSA, but again he developed a rash and pruritis. The onset of the symptoms were delayed, and the pruritis was very mild. He received Benadryl at a dose of 50 mg iv and the rash disappeared soon after the infusion was completed. The next day he was premedicated with 50 mg of Benadryl iv, but the erythema recurred and became generalized during the infusion. On the 4th and 5th days he was premedicated with 100 mg of hydrocortisone iv 4 hours before and at the start of the infusion of AMSA. The skin reaction was almost completely blocked, with only small patches of erythema noted. His pruritis was controlled with Benadryl, and he did not develop nasal congestion.

### DISCUSSION

The above case represents the only hypersensitivity reaction to AMSA seen in > 100 patients treated for acute leukemia (1,3) at this institution. This allergic reaction is rare and routine premedication is therefore not necessary in patients receiving AMSA. Although no life-threatening systemic symptoms were encountered, the possibility that this may occur should be kept in mind. In view of the ease of controlling the reaction seen, its occurrence should not lead to discontinuation of treatment with AMSA, but rather to routine premedication in a sensitive patient.

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Sydney Welt
Claudia Dellaquila
Zalmen A. Arlin*

Hematology/Lymphoma Service
Memorial Sloan-Kettering Cancer Center
1275 York Ave
New York, NY 10021

November 5, 1980

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*To whom reprint requests should be sent.

### *Cisplatin Treatment of Cutaneous T-Cell Lymphoma*

Surface marker studies have helped define mycosis fungoides and the Sézary syndrome as cutaneous T-cell lymphomas (1). Cutaneous lymphomas in early stages of disease respond to multiple treatment modalities. However, once disease has spread to visceral organs, there is no evidence that any mode of therapy has prolonged survival (2). Novel forms of systemic chemotherapy have been used in this center for patients with stage II–IV mycosis fungoides in an attempt to prolong survival (3).

Two patients refractory to all previous chemotherapy were treated weekly with a dose of 20 mg/m$^2$ of cisplatin. Both had complete clearing of all lesions by 2 weeks which persisted for up to 10 weeks.

The patients described were treated weekly with cisplatin after having failed other modes of topical and systemic therapy. The rationale for our use of this agent was based on our experience using cisplatin as a single agent in diffuse histiocytic and Hodgkin's lymphomas resistant to all other forms of conventional radiation therapy or chemotherapy. Dramatic short-term (< 4 months) palliative benefit has been provided by this single-agent therapy.

The drug can be delivered on an outpatient basis. Baseline audiometry, serial measurement of renal functions (creatinine, creatinine clearance, and BUN), and hematologic cell counts were performed to follow previously described toxic effects of the drug. Despite marked effectiveness in the resolution of ulcerative and plaque lesions in patient 1, administration of cisplatin was discontinued with the onset of nervous system abnormalities. Our concern was that the cisplatin was responsible for the change in neurologic status. Cisplatin was not detectable in the cerebrospinal fluid. This is in concurrence with standard distribution studies (4). Berman and Mann (5) have recently described seizures and cortical blindness in a 30-year-old man being treated with cisplatin for a stage II embryonal cell carcinoma of the testis. In their patient, cisplatin levels in the cerebrospinal fluid were nearly identical to those found in the serum. Post-mortem examination of the brain in patient 1 revealed an overgrowth of neurofibrillary tangles and axonal degeneration. Atomic absorption analysis of brain tissue from putamen and Ammon's horn for platinum levels are in progress (A. Prestayko, Bristol Laboratories, Syracuse, NY, personal communication).

Current standards of treatment for advanced cutaneous T-cell lymphoma at this center use 3000–3600 rads of total-skin electron-beam therapy, followed by 6 months of pulse chlorambucil, doxorubicin, and methyl-CCNU in an attempt to eliminate all tumor burden.

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Gary Witman*
Ed Cadman
Irwin Braverman

Yale University School of Medicine
New Haven, CT 06510



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**Reprint requests to:* Gary Witman, MD, Clinical Investigations Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Landow Bldg, Rm 4A18, National Institutes of Health, Bethesda, MD 20205.


### *Proper Analysis of Clinical Trials for Malignant Glioma*

There are serious flaws in the paper by Chin et al (1) on the use of radiotherapy with and without BCNU or methyl-CCNU for the treatment of malignant gliomas after surgery. The paper reports the experience at the University of Kentucky Medical Center, which had been a participating institution in several protocols of the Brain Tumor Study Group (BTSG). Some of the criticisms of this paper in part represent judgments as to correct methods of analysis, although we are confident that most researchers skilled in the analysis of clinical trials will agree with our statements. However, there can be no argument on misstatements of fact. It is false to state, as the authors do, that "this study was a controlled, prospective, randomized clinical trial and 115 patients were entered into the protocol program between 1966 and 1977" and "the protocol consisted

of. . .six arms." The six treatment groups described in the paper relate to two consecutive trials (BTSG 69–01, conducted in 1969–1972, and BTSG 72–01, conducted in 1972–1975), each consisting of four arms, two of which were nominally the same across studies (2,3). The protections against bias conferred by the structure of a concurrent randomized trial are not applicable here, and it is misleading to imply otherwise. Any patients included from the years 1966–1968 represented a separate clinical trial evaluating mithramycin; the relevance of patients studied during this interval to the present paper is unclear. Patients studied from 1976 to 1977 would have been entered in yet a fourth clinical trial with only one of the four arms being the same as those in the present paper; it is not clear if patients from this single arm were included in the results. If they were, it only compounds the problem.

The authors state that 28 patients "who received no more than two courses of chemotherapy" were excluded from evaluation. Because no such exclusion criterion applies to patients who were not assigned to chemotherapy, a bias is introduced into treatment comparisons. Furthermore, it is not clear exactly how the exclusion criterion was applied, since the third course of chemotherapy could not start until Week 13 at the earliest (based on 6–8 weeks/course) and yet the paper cites deaths in some chemotherapy groups prior to this point.

The authors mention the imbalances in prognostic factors that exist in their data, yet no attempt is made to account for them or perform adjusted analyses. For example, the percents of lower grade tumors in the two groups receiving methyl-CCNU were 40% and 44%, while for the other four groups they ranged from 20% to 31%. No mention is made of performance status at the time of randomization, known to be an important prognostic factor. Imbalances in prognostic factors are possibly more important than differences between the treatments being compared. In fact, it may be that greater imbalance was created by the exclusion of some patients, as noted above. Such patient heterogeneity becomes particularly important in dealing with very small sample sizes. Three of the six groups reported in the paper consisted of five, nine, and ten patients. To compare median survival times in such small heterogeneous populations is to risk basing medical knowledge on random noise. This is true even if there was no concern for bias due to selecting subsets of patients in an improper manner. The combined effects of variation in small samples plus bias due to subset selection can explain why the median survival times differed from those in the overall BTSG studies.

The discussion of modal survival in such small samples is nonsense. As Armitage (4) states, "some distributions will appear to have more than one mode owing to the inevitable random fluctuations of small numbers . . . although we should be inclined to pay no great attention to these." He also states that the mode "is not widely used in analytical statistics, mainly because of the ambiguity in its definition as the fluctuations of small frequencies are apt to produce spurious modes." The fact that two of the five patients in the BCNU group happened to die in the same 8-week interval does not define a quantity that is meaningful either statistically or medically. Figures 2 and 3, which show the distribution of survival times, are by themselves potentially useful, but even these are open to question since the sample sizes implicit in the figures do not match the counts given elsewhere. For example, 11 deaths are indicated in figure 3 for the methyl-CCNU plus radiotherapy group compared to ten in the tables; only 11 deaths are apparent in figure 2 for the surgery-alone group compared to 12 in the tables. This analysis is influenced greatly by the choice of interval, and therefore the values presented for heights and locations of "modes" have little meaning.

The authors conclude by stating that patients receiving radiation therapy and methyl-CCNU "had a significantly increased survival . . . compared to all other groups," yet nowhere in the paper is there evidence for the significance of the difference between BCNU plus radiotherapy compared to methyl-CCNU plus radiotherapy.

Although the patients studied are the same as some of those reported in BTSG publications, the findings do not represent bona fide BTSG results. Analyses of the complete studies (69–01 and 72–01) have been published (2,3), although Chin et al (1) fail to cite the first of these reports. In the analysis (3) of the overall results of BTSG 72–01, BCNU plus radiotherapy did not differ significantly from methyl-CCNU plus radiotherapy in a properly randomized comparison. Estimates of median survival for these treatment groups were 51 and 42 weeks, respectively.

Although publication of the results of a single participant's experience can be a useful exercise under proper conditions, the publication of small studies, misleadingly described and improperly analyzed, only renders a disservice to the medical community. This harsh assessment is justified by the specifics of the paper in question. In analogy to the maxim "let the buyer beware," we advise "let the reader beware" of conclusions built on very shaky foundations.

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Sylvan B. Green*
David P. Byar
Thomas A. Strike
Michael D. Walker[1]


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*Editor's note: A letter of reply will appear in a later issue of* Cancer Treatment Reports.


[1]Biometry Branch, Division of Cancer Cause and Prevention, National Cancer Institute (*S. B. Green* and *D. P. Byar*); Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute (*T. A. Strike*); and Stroke and Trauma Program, National Institute of Neurological and Communicative Disorders and Stroke (*M. D. Walker*); Bethesda, MD.

**Reprint requests to:* Dr. Sylvan B. Green, Biometry Branch, National Cancer Institute, Landow Bldg, Rm 5C09, National Institutes of Health, Bethesda, MD 20205.

## *Dianhydrogalactitol in the Treatment of Children With Primary Brain Tumors*[1]

Dianhydrogalactitol (DAG), the diepoxide of dibromodulcitol, has produced marked tumor growth inhibition and increases in animal survival in a number of experimental tumor systems (1), including an intracerebral tumor in mice (2). It is regarded as an alkylating agent from studies of the chemical activity and cross-resistance to alkylating agents (3,4), but the exact mechanism of action is still not clear and cannot be explained by alkylation alone. Because of its ability to cross the blood-brain barrier and its demonstrated beneficial effect on several patients in phase I trials, including one patient with cerebral metastases from a small cell lung cancer (5), a phase II study of DAG was instituted in children with a variety of pediatric malignancies at the Childrens Hospital of Los Angeles.

Twelve children believed to have recurrent or progressive primary brain tumors were entered in this study (table 1). All children received prior therapy, including radiation therapy, chemotherapy, and surgical resection when possible. The drug was given in a dose schedule of 37.5 or 50 mg/m² twice weekly,

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TABLE 1.—Summary of patients treated with DAG

| Patient No. | Diagnosis | Prior therapy* | Diagnosis of relapse† | Age (yrs) | Total dose (mg) and time | Results‡ | Toxicity |
|---|---|---|---|---|---|---|---|
| 1 | Medulloblastoma | Subtotal resection, 5250r, CCNU, VCR, PRD | SX, LP | 6 | 434, 3 mos | Progression | Pancytopenia × 2 mos; sepsis |
| 2 | Brain stem glioma | Subtotal resection, 6060r, CTX, VCR, PBZ, PRD | SX, CT, RN, LP | 9 | 120, 6 wks | Progression, death from sepsis | Pancytopenia × 7 wks |
| 3 | Brain stem glioma | Biopsy, 5000r, CCNU, VCR, PBZ, PRD | SX, CT, RN, LP | 9 | 480, 6 wks | Progression | Pancytopenia × 9 wks |
| 4 | Medulloblastoma | Subtotal resection, 5400r, VCR, CCNU, PBZ, CDDP | LP§ | 5 | 670, 20 mos | Asymptomatic, NED | Pancytopenia × 10 wks |
| 5 | Medulloblastoma | Subtotal resection, 6170r, VCR, PRD, CCNU, PBZ | LP§ | 8 | 1302, 19 mos | Asymptomatic, NED | Pancytopenia × 2 mos |
| 6 | Oligodendroglioma | Subtotal resection × 3, 5850r, VCR, CCNU, PBZ, PRD | CT, RN, LP | 14 | 1076, 7 mos | Progression | Pancytopenia × 2 mos |
| 7 | Medulloblastoma | Subtotal resection, 4680r, VCR, CCNU, PBZ | LP | 15 | 390, 3 mos | Progression | Pancytopenia × 2 mos |
| 8 | Medulloblastoma | Subtotal resection, 5800r, VCR, CCNU, PRD, PBZ | LP§ | 10 | 992, 16 mos | Asymptomatic, NED | Pancytopenia × 3 mos |
| 9 | Hypothalamic glioma | Biopsy, 4600r, VCR, CCNU, PBZ, PRD | CT§ | 14 | 533, 5 mos | Death from sepsis, NED | Pancytopenia × 2½ mos; sepsis, shock |
| 10 | Parietal astrocytoma | Subtotal resection, 5250r, VCR, CCNU, PBZ | CT, LP | 10 | 425, 5 mos | Progression | Pancytopenia × 6 wks |
| 11 | Brain stem glioma | Subtotal resection, 5230r, VCR, PRD, CCNU, PBZ | CT§, LP§, RN§ | 7 | 225, 6 mos | Asymptomatic, AML 1 yr later | Pancytopenia × 1 mo; neutropenia × 4 mos |
| 12 | Brain stem glioma | Subtotal resection, 5500r, VCR, CCNU, PRD, PBZ | SX, CT, RN | 4 | 341, 5 mos | Progression and spinal seeding | Pancytopenia × 7 wks |

*r = rad, VCR = vincristine, PRD = prednisone, CTX = cyclophosphamide, PBZ = procarbazine, and CDDP = cisplatin.
†SX = symptomatic, LP = lumbar puncture, CT = computerized tomography, and RN = radionuclide scan.
‡NED = no evidence of disease, and AML = acute myelocytic leukemia.
§False-positive study.

with modification for toxicity as needed. Four symptomatic patients and three patients who were asymptomatic at the time of disease recurrence all showed signs of disease progression and died of the underlying disease. Five asymptomatic patients were treated with DAG following false-positive diagnostic tests, most commonly an isolated finding of suspicious cells in the cerebrospinal fluid, and none developed neurologic symptoms despite discontinuation of the drug and persistence of the abnormal test. Severe bone marrow suppression, most commonly profound granulocytopenia, was universally observed and required withholding of the drug and subsequent dose modification. Two patients, one who was free of disease, died of sepsis during prolonged periods of drug-induced granulocytopenia. Another patient became severely pancytopenic after 6 months of DAG treatment and ultimately developed acute myeloblastic leukemia, but a direct causal relationship cannot be inferred due to prior treatment with other potentially carcinogenic agents (including procarbazine and CCNU, as well as radiation).

DAG was found to be a highly toxic drug that causes prolonged bone marrow suppression, and, at the dose schedule used, was of no value in the treatment of primary brain tumors in children.

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Mark E. Wienblatt
Jorge A. Ortega*
Gussie R. Higgins
Stuart E. Siegel

Department of Pediatrics,
University of Southern California School of Medicine
and
Division of Hematology-Oncology
Childrens Hospital of Los Angeles
Los Angeles, CA



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*Reprint requests to:* Jorge A. Ortega, MD, Division of Hematology-Oncology, Childrens Hospital of Los Angeles, 4650 Sunset Blvd, Los Angeles, CA 90027.


### *5-FU, Methotrexate, and Mitomycin in Advanced Colorectal Adenocarcinoma: A Phase II Study*

Thirty-one patients with advanced colorectal adenocarcinoma received chemotherapy with 5-FU, methotrexate, and mitomycin (FMM) according to the following scheme: 600 mg/m² of 5-FU and 30 mg/m² of methotrexate iv on Days 1, 8, 29, and 36, and 10 mg/m² of mitomycin iv on Day 1. The cycle was repeated every 8 weeks. For patients > 65 years old, the doses were reduced by one third. Twenty-four patients had visceral, primarily hepatic (21 patients), metastases; five had pelvic disease; and two had retroperitoneal disease. Twelve patients were previously treated with radiotherapy (five patients), chemotherapy (four), or both (three). The median pretreatment serum CEA titer was 50 ng/ml (range, 25–380).The treatment did not result in any complete or partial responses. Five patients achieved a minimal response, defined as a > 25% but < 50% reduction in measurable tumor or a 25% decrease in CEA titer with > 20% improvement in

Table 1.—Relationship between pretreatment characteristics and response in patients receiving FMM for colorectal adenocarcinoma

| Characteristic* | Patients with— Minor response (MR) or stable disease (S) | | No response |
|---|---|---|---|
| Age (yrs) | | | |
| Median | 66 | | 60 |
| Range | 29–77 | | 44–68 |
| Sex | | | |
| Males | 5 | | 7 |
| Females | 13 | | 6 |
| Karnofsky performance score (%) | | | |
| Median | 80 | | 50 |
| Range | 40–90 | | 20–90 |
| Prior treatment | | | |
| No | 10 | | 8 |
| Yes | 8† | | 5 |
| CEA (ng/ml) | MR | S | |
| Median | 8.5 | 50 | 76 |
| Mean | 13.0 | 85 | 65 |
| Range | 5–30 | 18–380 | 25–100 |
| Dukes' classification | | | |
| B + C | 3 | | 7 |
| D | 14‡ | | 5‡ |
| Metastases | | | |
| Visceral | 13 | | 9 |
| Nonvisceral | 5 | | 4 |

*Unless otherwise specified, values = No. of patients.
†No minor responses.
‡Not documented in an additional patient.

performance status (1). In 13 patients, the disease stabilized. Minor responses lasted a median of 1 month (range, 2–13), and stable disease lasted a median of 5 months (range, 2–16). In 13 patients, disease progression continued. The group median survival from the start of FMM treatment was 6 months (range, 2–21+). Patients with minor response or stable disease lived 7.5 months (range, 4–21+) whereas nonresponders lived only 4 months (range, 2–8+). The statistical difference in survival between these two groups is significant ($P = 0.004$) (2). Six of 18 patients with minor response or stable disease survived > 12 months; however, only two of these survived 2 years. Table 1 lists several pretreatment characteristics which, in some instances, correlated with response. High performance status, class D disease (Dukes' classification) at presentation, and female gender were favorable characteristics. The patients who achieved minor responses had had no treatment prior to FMM and a relatively low initial CEA titer. The main toxic effect was transient myelosuppression. Median leukocyte and platelet count nadirs were 3000/mm³ (range, 500–14,000) and 70,000/mm³ (range, 6000–210,000), respectively. During administration of 135 FMM courses there were 14 hospitalizations: ten for leukopenia and fever and four due to bleeding. One drug-related death occurred. Mild to moderate stomatitis was experienced by 11 patients. Toxicity was unrelated to age.

We conclude that the FMM regimen is associated with acceptable toxicity but has no major impact on the natural course of advanced colorectal cancer (3). A subgroup of previously untreated patients with a good performance status may, however, benefit from this combination chemotherapy and further trial in this selected group seems warranted.

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Haim Biran
Aaron Sulkes
Shoshana Biran

Department of Radiation and Clinical Oncology
Hadassah University Hospital
Jerusalem, Israel



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### *Chemoimmunotherapy (DTIC and Corynebacterium parvum) for Disseminated Malignant Melanoma*

Chemoimmunotherapy with DTIC or other drugs and *Corynebacterium parvum* has been reported to potentiate duration of remission and survival of responders compared to chemotherapy alone (1), but this has been disputed (2,3).

In the present study, 37 evaluable patients with metastatic malignant melanoma (table 1) were treated with DTIC (250 mg/m² iv daily × 5) and *C. parvum* (Burroughs-Wellcome) (0.7, 1.4, 2.8, 3.5, and 3.5 mg iv, respectively, on each of the 5 days of treatment) repeated every 4 weeks. The dose of *C. parvum* was increased daily if the patient's temperature remained < 102°F, was kept the same for patients with temperatures of 102°F–104°F, and was halved if the fever exceeded 104°F. Fever of ≥ 102°F was treated with antipyretics, and fever of ≥ 104°F was treated also with 100 mg of hydrocortisone iv.

Lymphocyte stimulation in vitro was studied with phytohemagglutinin (PHA), Varidase, purified protein derivative, mumps vaccine, or *Candida* extract in short-term lymphocyte cultures set up by a whole-blood method (4).

Three patients had complete regression, while receiving chemoimmunotherapy which was continued for 1 year. This lasted 14 months in a man with multiple subcutaneous, pulmonary, and osseous metastases. Two women with soft tissue metastases

Table 1.—Characteristics and responses of patients

| Characteristic | No. of patients |
|---|---|
| No. of patients | 37 |
| Sex | |
| Male | 24 |
| Female | 13 |
| Age in yrs | |
| Median: 51 | |
| Range: 29–75 | |
| Previous chemotherapy | |
| Yes | 6 |
| No | 31 |
| Sites of measurable disease | |
| Soft tissue | 15 |
| Viscera | 22 |
| Hematopoietic toxicity | |
| Wbc count < 2500/mm³ | 1 |
| Platelet count < 75,000/mm³ | 1 |
| Response | |
| Complete | 3 |
| Partial | 3 |
| Total | 6 (16%) |

remain well at 3½ and 4 years, respectively, after complete regression. Three patients, one with visceral and two with distant lymph node metastases, had partial (> 50%) regressions of 15, 8, and 5 months' duration, respectively. Six patients had stabilization of 5–7 months' duration (median, 6). Twenty-five patients had disease progression after one to three courses of chemotherapy.

One patient with hyperpyrexia developed hypotension which was promptly corrected with iv hydrocortisone.

In vitro lymphocyte blastogenic response to PHA was depressed in 14 of 29 patients. There was no correlation between pretreatment PHA or antigen response and tumor response to chemoimmunotherapy.

The observed objective response rate of 16% in this series is not substantially different from that achieved with DTIC alone (5). The duration of response appears to be longer in our patients than the commonly reported duration of 5–6.7 months for DTIC alone (5), but the difference with DTIC-treated patients was not statistically significant.

*C. parvum* may possibly have a beneficial effect in causing tumor stabilization or regression by nonspecifically stimulating the immune system, increasing tolerance to chemotherapy, and/or increasing effectiveness of chemotherapy in the presence of temperature elevation. However, until a better understanding and quantitation of the role of the host's immune response and temperature elevation in the control of tumor growth is achieved, chemoimmunotherapy with *C. parvum* is not likely to produce major therapeutic advances over those attained by systemic chemotherapy alone.

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C. P. Karakousis*
M. S. Didolkar[1]
*Department of Surgical Oncology*
T. Han[2]
*Department of Medical Oncology*

Roswell Park Memorial Institute
666 Elm St
Buffalo, NY 14263



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[1]*Present address:* Division of Oncology, Department of Medicine, University of Maryland, Baltimore.


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*To whom reprint requests should be addressed.

# ANNOUNCEMENTS

## Clinical Conference on Leukemia and Lymphoma

***Toronto, Canada, November 7, 1981***

The Toronto Chapter of the Ontario Oncology Association for Health Professionals is planning a clinical conference on leukemia and lymphoma to be held November 7, 1981, at Mount Sinai Hospital in Toronto. The cost, including lunch and coffee, is $25 for members and $30 for nonmembers. *For more information, contact*: Jennifer McNaught, 175 Trudelle St, Unit 55, Scarborough, Ontario, Canada (416–486-4800).

## Management of Advanced Cancer

***Amarillo, Texas, November 12–14, 1981***

The "Management of Advanced Cancer" will be the topic of the inaugural scientific symposium of the Don and Sybil Harrington Cancer Center to be held November 12–14, 1981, in Amarillo, Texas.

Joseph Bertino, MD, keynote speaker for the conference, will present a two-part lecture entitled "Pharmacological Strategies for the Treatment of Advanced Cancer." Other topics to be discussed include support programs for patients and families, the management of advanced solid tumors, and the treatment of hematologic malignancies.

Invited speakers are J. Cox, D. Rockoff, R. Benjamin, J. Mendelsohn, D. Von Hoff, G. Phillips, C. A. Coltman, C. D. Bloomfield, L. Lessin, R. J. Mayer, E. M. Copeland, H. L. Borison, K. M. Thaney, S. B. Lansky, D. Ihde, and T. Wharton.

The symposium, which is cosponsored by Texas Tech University Health Sciences Center, will be chaired by Drs. Phillip Periman and Edwin D. Savlov.

*For additional information, write or call*: Mrs. Janie Brown, Medical Directors Office, Don and Sybil Harrington Cancer Center, 1500 Wallace Blvd, Amarillo, TX 71906 (806–353-3571).

## Current Concepts in Cancer Therapy

***Saint Louis, Missouri, December 10–12, 1981***

A program entitled "Current Concepts in Cancer Therapy" will be held December 10–12, 1981, in Saint Louis, Missouri. The Program Chairman is Marc K. Wallack, MD. The conference is being presented by the Section of Surgical Oncology, Department of Surgery, Washington University in Saint Louis and the Missouri Chapter of the American Cancer Society. The American Medical Association, American Academy of Family Physicians, and American Osteopathic Association have approved 19 credit hours for this program.

*For information, contact*: Office of Continuing Medical Education, Washington University School of Medicine, Box 8063, 660 S Euclid Ave, Saint Louis, MO 63110 (314–454-3873).

## Childhood Cancer: Impact on Family

***Brooklyn, New York, January 29–30, 1982***

A major symposium on how families cope with cancer in children and adolescents will be held January 29–30, 1982, at Downstate Medical Center, Brooklyn, New York. Speakers will include Drs. Denis R. Miller, Jan Van Eys, Ida Martinson, Adolph E. Christ, David M. Kaplan, Bernard H. Fox, and Mrs. Grace Christ, MSW. This program has been approved for 12 hours of AMA Category I credit. *For further information, contact*: Dr. Kalman Flomenhaft, Downstate Medical Center, Box 32, 450 Clarkson Ave, Brooklyn, NY 11203 (212–270-1436).

## Roswell Park Memorial Institute Continuing Education Programs

***Buffalo, New York, February—June 1982***

Roswell Park Memorial Institute is conducting monthly continuing education programs in oncology for community practitioners to disseminate information on newest methods in the diagnosis and treatment of cancer. Each monthly seminar is organized by a different department of the Institute covering a different clinical specialty. These seminars qualify for Category I credit toward the AMA Physician's Recognition Award, prescribed credit by the American Academy of Family Physicians, and Category 2-D credit by the American Osteopathic Association. There is a $25.00 registration fee for one-day seminars and a variable fee for longer programs. The seminars are co-sponsored by the Cancer Control Program of the Institute and the American Cancer Society, New York State Division and Erie County Unit.

The seminars are scheduled as follows: Soft Tissue Tumors, *February 11, 1982* (chairperson: Dr. E. Douglas Holyoke); What's New in Urologic Oncology, *March 4, 1982* (chairperson: Dr. J. Edson Pontes); Tumors Involving the Skin, *April 1, 1982* (chairperson: Dr. Ole Holtermann); Controversies in the Management of Childhood and Adolescent Cancer, *May 6, 1982* (chairperson: Dr. Arnold Freeman); Cancer of the Colon-Rectum, *June 5, 1982* (chairperson: Dr. Arnold Mittelman).

*Inquiries should be sent to*: Gayle Bersani, RN, Cancer Control Coordinator, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263 (telephone: 716–845-4406).

## First Keystone Symposium on Hematologic Malignancies

***Keystone, Colorado, March 14–21, 1982***

A multidisciplinary faculty from the University of Arizona Cancer Center will present the First Keystone Symposium on Hematologic Malignancies to be held at Keystone, Colorado, March 14–21, 1982. Registration is limited and the deadline for hotel registration is November 15, 1981.

*For further information contact*: Stephen E. Jones, MD, 4 West, Cancer Center, University of Arizona, Health Sciences Center, Tucson, AZ 85724 (602–626-6372).

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